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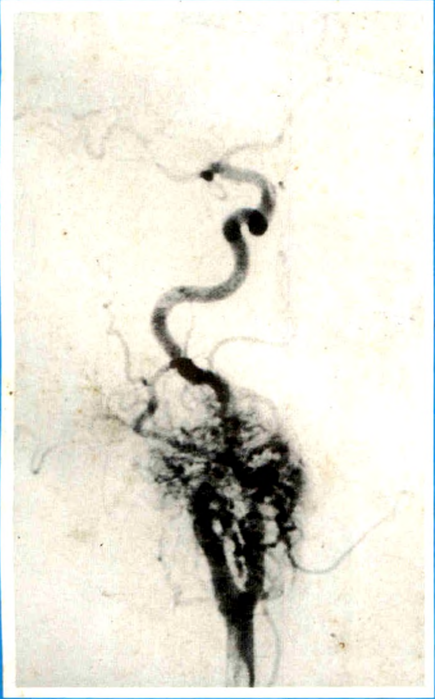

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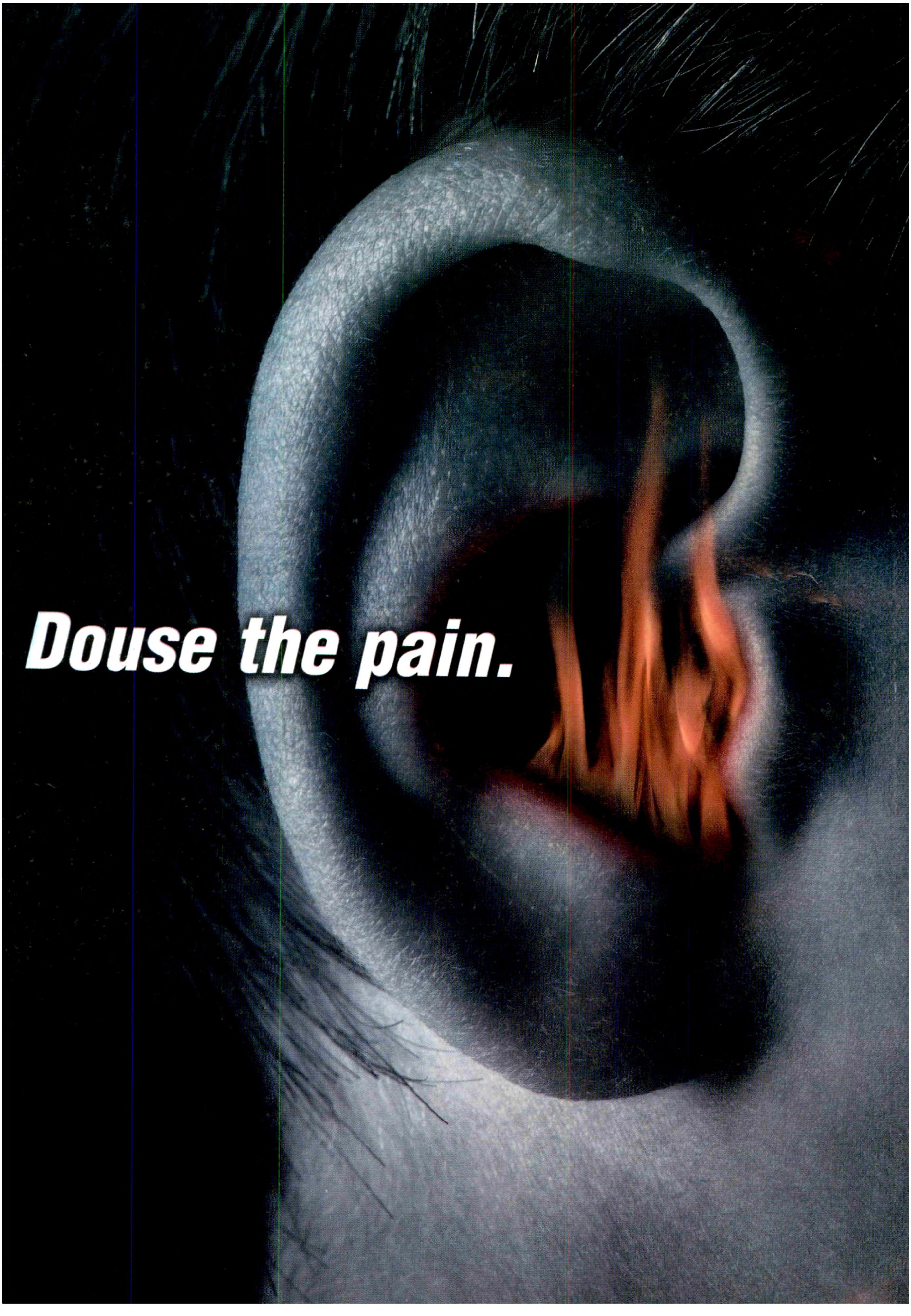
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Between 1981 and 1994, 34 patients with squamous cell carcinoma of the supraglottis were treated by transoral carbon dioxide laser resection, 12 of them palliatively. Additional treatment included neck dissection in 21 patients and radiotherapy in 24 patients. The 3-year overall survival was 62%, and the actuarial survival 80%. The overall survival for T1 and T2 tumors was 71%, and that for T3 and T4 tumors was 47%. The overall 3-year survival for the early stages, I and II, was 88%, and that for the advanced stages, III and IV, was 50%. These results are comparable to the outcome after conventional open partial resection. Given the significantly lower morbidity (only 7 patients required tracheostomy), we do not observe an age limit anymore. The transoral method can be recommended as curative treatment in T1 and T2 tumors and in selected T3 and T4 tumors in concert with neck dissection and/or radiotherapy. In patients with advanced inoperable tumors, laser surgery is an excellent alternative to tracheostomy and palliative radiotherapy. Prerequisites for successful application of the transoral carbon dioxide laser resection are adequate resection techniques.

KEY WORDS — carbon dioxide laser surgery, supraglottic cancer.

INTRODUCTION

It was Alonso[1] who in 1947 described horizontal supraglottic partial resection as a surgical treatment of supraglottic carcinoma. His method took several decades to gain widespread recognition. Myers and Alvi[2] believe that the reason for this was that the time was not ripe for this idea to be accepted, as it seemed to upset the traditional and deeply rooted principles of cancer surgery. The history of the delayed acceptance of external supraglottic partial resection now seems to be repeated by endoscopic partial resection. This is even more regrettable, since from a functional point of view, endoscopic partial resection is greatly superior to open partial resection. The reluctant acceptance of endoscopic partial resection is most likely a reflection of skepticism regarding oncologically safe resection margins as they have been proven for open partial resection. The purpose of this study was to show that the oncological results of this new method compare favorably to those of other surgical methods.

PATIENTS AND METHODS

Between January 1, 1981, and December 31, 1994, 34 previously untreated patients (28 men and 6 women) with squamous cell carcinoma of the supraglottis were treated at the Department of Otorhinolaryngology–Head and Neck Surgery of the University of Kiel. The average age was 62 years (range 44 to 89 years). Eight patients (23%) were older than 65 years. Follow-up data were obtained for all patients until the time of death or until at least March 1997 (Tables 1 and 2). The 1987 version of UICC-AJCC (Union Internationale Contre le Cancer–American Joint Committee on Cancer) staging was used. Table 3 shows the T and N classifications of the patients. It refers to surgical and histopathologic staging concerning T status. The N status was determined histopathologically when a neck dissection was performed. In other cases, the staging is based on ultrasound findings. In Table 4, the classification by stages is represented. None of our patients had distant metastasis at primary diagnosis. Nodal disease was already present at first diagnosis in 50% of the cases. Initially, the indication for laser surgery was palliative in 12 patients (Tables 1 and 2). The criteria for a palliative resection were the patient's reduced medical status and advanced age in 4 cases, a second primary dominating the prognosis in 1 case, a tumor that could not have been completely removed by a laryngectomy in 1 case, and refusal of laryngectomy by 6 patients. One patient refused laryngectomy, neck dissection, and radiotherapy. These 12 patients were included in the analysis, since 7 of them remained free of local recurrence.

In 21 patients, a neck dissection was performed: in 9 patients in 1 procedure with the laser surgery, and in 12 patients about 7 days after the laser surgical

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TABLE 1. CHARACTERISTICS AND OUTCOME OF 24 PATIENTS WITHOUT LOCAL RECURRENCE

Pt No.	*Age, Sex*	*TNM*	*Intention*	*Tumor Location*	*Therapy; Reason for Palliative Treatment*	*Tracheostomy*	*Nasogastric Tube*	*Complications*	*Follow-up*	*Metastatic Recurrence*	*Second Primary*	*Death Not Related to Primary Tumor Location*
1	69, M	T1N1M0	Cura	Supra epi	Laser, unilat ND, RT		5 d		3 y 8 mo		Prostate cancer	Cardiopulmonary disease 3 y 8 mo
2	79, M	T1N0M0	Pall	Supra epi	Laser, RT; age		5 d		8 y 5 mo			
3	61, M	T1N1M0	Cura	Supra epi	laser, bilat ND, RT	56 d			6 y 3 mo		Oropharyngeal cancer, no evidence of disease	
4	51, F	T1N0M0	Cura	Supra epi	Laser	Preop (bilat RLN palsy)			5 y 8 mo			
5	70, M	T1N2bM0	Cura	Supra epi	Laser, unilat ND, RT				2 y 7 mo			Coronary heart disease 2 y 7 mo
6	55, M	T2N0M0	Cura	Supra epi, ary fold	Laser, unilat ND, RT	7 d	13 d		7 y			
7	51, M	T2N0M0	Cura	Supra + infra epi	Laser, bilat ND, RT		7 d		7 y 11 mo		Lung cancer, alive with 2nd primary	
8	74, M	T2N1M0	Pall	Infra epi, ven fold	Laser; refused LE, ND, RT	Decades preop (diphtheria)	4 mo	Persistent dysphagia, LE after 1 y 4 mo	4 y 11 mo		Adenocarcinoma of submandibular gland, palatine arch cancer, no evidence of 2nd or 3rd primary	
9	66, M	T2N0M0	Cura	Supra + infra epi	Laser, RT				5 y 8 mo			Coronary heart disease 5 y 8 mo
10	76, M	T2N1M0	Cura	Supra + infra epi	Laser, unilat ND, RT	Permanent	7 mo	Persistent dysphagia	7 y			
11	63, M	T2N1M0	Cura	Supra epi, ary fold	Laser, unilat ND, RT		7 d		6 y 3 mo		Tongue cancer, no evidence of 2nd primary	
12	63, F	T2N0M0	Cura	Infra epi, ven fold	Laser, RT				2 y		Lung cancer, dead of 2nd primary	Lung cancer 2 y
13	44, M	T2N0M0	Cura	Supra + infra epi	Laser, bilat ND		22 d	Hemorrhage	3 y 7 mo			
14	71, F	T2N0M0	Cura	Infra epi, ven fold	Laser, unilat ND		5 d		3 y 7 mo			
15	59, M	T3N0M0	Cura	Infra epi, ven fold	Laser, bilat ND		6 d		4 y 4 mo			
16	89, M	T3N1M0	Pall	Epi, vent + ary fold	Laser, unilat ND, RT; age		5 d		1 y 6 mo			Coronary heart disease 1 y 6 mo
17	53, F	T3N0M0	Cura	Epi, vent + ary fold	Laser, unilat ND		5 d		7 y 7 mo	Lung		Coronary heart disease 7 y 7 mo
18	61, M	T3N0M0	Pall	Infra epi, ven fold, VC	Laser; 2nd primary		6 d		1 y 7 mo		Lung cancer	Coronary heart disease 1 y 7 mo
19	55, M	T3N0M0	Pall	Epi, ven fold, VC	Laser, unilat ND, RT; refused LE		15 d	Hemorrhage, dysphonia	2 y		Lung cancer, dead of 2nd primary	Lung cancer 2 y
20	62, M	T3N1M0	Cura	Epi, ven fold, VC	Laser, unilat ND, RT		6 d		3 y 8 mo		Bladder cancer, alive with disease	
21	47, M	T3N2bM0	Cura	Supra + infra epi, ven + ary fold	Laser, bilat ND, RT		109 d	Dysphagia	3 y	Neck		
22	45, F	T4N1M0	Pall	Supra + infra epi, ven + ary fold	Laser, unilat ND, RT; refused LE	75 d	13 d		6 y	Lung, brain		
23	65, M	T4N0M0	Pall	Supra + infra epi, BOT	Laser, RT; refused LE		1 d	Dysphonia	3 y 4 mo	Lung, brain, death after 3 y 4 mo		Distant metastases 3 y 4 mo
24	67, M	T4N0M0	Cura	Supra + infra epi, BOT	Laser, bilat ND, RT		11 d		3 y 3 mo			Coronary heart disease 3 y 3 mo

Cura — curative, supra epi — suprahyoid epiglottis, ND — neck dissection, RT — radiotherapy, pall — palliative, RLN — recurrent laryngeal nerve, ary fold — aryepiglottic fold, infra epi — infrahyoid epiglottis, ven fold — ventricular fold, LE — laryngectomy, epi — epiglottis, VC — vocal cord, BOT — base of tongue.

removal of the local tumor. In 24 patients, radiotherapy was applied. The dosage was 54 Gy for the lymphatics and 60 Gy for the primary. The indications for postoperative radiotherapy were histologically positive lymph nodes in 12 patients, positive tumor margins in 2 patients (Nos. 6 and 7), and tumor size

TABLE 2. CHARACTERISTICS AND OUTCOME OF 10 PATIENTS WITH LOCAL RECURRENCE

Pt No.	*Age, Sex*	*TNM; Intention*	*Tumor Location*	*Therapy; Reason for Palliative Treatment*	*Tracheotomy*	*Nasogastric Tube*	*Complications*	*Follow-up*	*1st Recurrence and Therapy*	*2nd Recurrence and Therapy*	*2nd Primary*	*Outcome*
25	56, F	T2N0M0; curative	Supra epi, ary fold	Laser				3 y 1 mo	Epi 1 y; laser, RT		Esoph cancer, DO 2nd primary	DO 2nd tumor, NE of disease 2 y 1 mo after recurrence
26	81, M	T2N2bM0; palliative	Supra + infra epi	Laser, RT; refused LE				2 mo			Lung cancer	DO lymph node metastases 2 mo after therapy, residual disease
27	61, M	T2N2cM0; curative	Supra + infra epi	Laser, bilat ND, RT	24 d	3 d	RT interrupted	2 y 10 mo	Epi, ary fold 1 y 9 mo; LE	BOT 7 mo		DO primary 6 mo after recurrence
28	78, M	T3N0M0; palliative	Epi, ary + ven fold	Laser, RT; poor medical status				1 y	Residual larynx 9 mo; LE			DO primary 3 mo after recurrence
29	61, M	T3N1M0; curative	Epi, preepi space	Laser, bilat ND, RT	9 mo	43 d		1 y 8 mo	Residual larynx 9 mo; LE	BOT 8 mo; chemo		DO primary 3 mo after recurrence
30	46, M	T4N1M0; curative	Epi, BOT	Laser, RT		11 d	RT interrupted	1 y 9 mo	BOT 1 y 3 mo; laser*			DO lymph node metastases 6 mo after primary recurrence
31	60, M	T4N2cM0; palliative	Epi, BOT	Laser, bilat ND, RT; tumor size		4 d		2 y 1 mo	Residual larynx 2 y; LE		Lung cancer, NE of 2nd primary	DO primary 1 mo after recurrence
32	52, M	T4N0M0; palliative	Epi, BOT, ary + ven fold	Laser, RT; refused LE		13 d		10 mo	BOT 8 mo			DO heart disease 2 mo after recurrence
33	49, M	T4N3M0; palliative	Epi, BOT	Laser; poor medical status	Permanent 5 d after therapy	8 d	Pneumonia	1 mo				DO lymph node metastases 1 mo after therapy, residual disease
34	54, M	T4N1M0; curative	Epi, BOT	Laser, bilat ND		2 mo	Hemorrhage	3 y 3 mo	BOT, HP 8 mo; LE, RT	BOT, tonsil 1 y 4 mo; chemo		Alive with disease 15 mo after recurrence

Supra epi — suprahyoid epiglottis, ary fold — aryepiglottic fold, epi — epiglottis, RT — radiotherapy, DO — dead of, NE — no evidence, infra epi — infrahyoid epiglottis, LE — laryngectomy, ND — neck dissection, BOT — base of tongue, ven fold — ventricular fold, HP — hypopharynx.

*Had metastatic recurrence in neck at 1 y 5 mo.

in 7 patients. In 3 patients, postoperative radiotherapy was applied instead of an elective neck dissection.

Intraoperative tracheostomy was applied in only 6 patients. One patient had to be tracheostomized 5 days after surgery due to pneumonia. An additional 2 patients had received a tracheostomy many years before: 1 for a postdiphtheric laryngeal stenosis in childhood, and the other for bilateral recurrent laryngeal nerve paralysis following thyroidectomy. A nasogastric tube was placed in 26 patients.

Operative Technique of Transoral Supraglottic Partial Laryngectomy. The operative procedures were performed under general anesthesia in orotracheal intubation. Closed laryngoscopes are not suitable for procedures in the supraglottic region. Spreadable endoscopes are necessary. We have modified the spreadable laryngoscope described by Weerda into a so-called supraglottiscope.[3,4] The endoscopes are equipped with suction tubes to evacuate the laser plume. Suction tubes were also added to the microsurgical forceps and graspers.[5] Modern micromanipulators with a spot size of 0.25 to 0.3 mm should be used with the carbon dioxide (CO_2) laser. The laser is only used as a cutting instrument, and not for vaporization of tumor. In using the continuous wave mode with a laser power of 1 to 2 W, the tissue can be divided layer by layer, as if a scalpel were used. The zone of coagulation at the border of the incision measures only 0.5 mm. The pathologist can evaluate the borders of the resection almost as well as if a scalpel were used.

Smaller tumors of the border of the epiglottis and tumors of the aryepiglottic fold are excised with the laser with a safety margin of at least 1 cm as an excisional biopsy (Fig 1). In cases of larger tumors of the suprahyoid epiglottis, the resection is facilitated by dividing the epiglottis[3-8] (Fig 2). Tumors extending over the midline are also divided by this incision. From the classic oncosurgical point of view, this is an unusual approach. It is only possible due to the cutting characteristics of the CO_2 laser. The resection of tumors extending toward the infrahyoid epiglottis must include the preepiglottic space (Fig 3). In these tumors, we also start with a vertical division of the epiglottis. The next incision is guided along the val-

TABLE 3. TN CLASSIFICATION OF ALL PATIENTS

	T1	*T2*	*T3*	*T4*	
N0	2	7	5	3	17
N1	2	3	3	3	11
N2a					
N2b	1	1	1		3
N2c		1		1	2
N3				1	1
	5	12	9	8	34

All patients were M0.

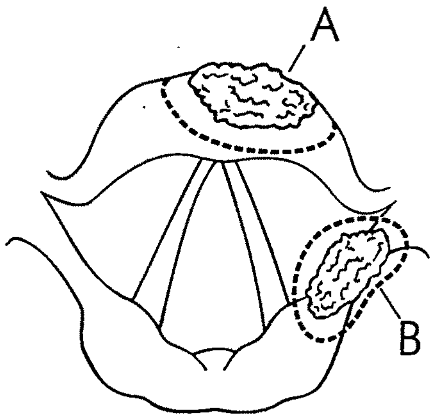

Fig 1. Laser resection lines of small carcinomas of border of epiglottis (A) and of aryepiglottic fold (B).

leculae until the hyoid is reached and then exposed. The incision goes caudally from the hyoid toward the hyothyroid membrane until the upper border of the thyroid cartilage is reached. From this point, the complete preepiglottic space is removed along the inner surface of the lamina of the thyroid cartilage, toward the anterior commissure of the vocal cords. False vocal cord tumors can also be resected transorally with the same technique. The false vocal cord can then be completely dissected from the thyroid cartilage along its inner surface, toward the sinus of Morgagni.

RESULTS

Local Control. No recurrence was observed in 24 patients (Table 1), including 7 of 12 patients treated with palliative intention. Local recurrence was found in 10 patients (Table 2), 5 of whom were initially treated with palliative intention.

Analysis With Regard to T Category. All 5 patients with T1 tumors (100%) remained free of local recurrence. Of this subgroup, 2 (40%) eventually incurred secondary malignancies. But no regional or distant metastasis was noticed in the follow-up.

Three (25%) of 12 patients with T2 tumors suffered from local recurrence. One of these patients (No. 25) was treated by laser surgery for his recurrence. He remained free of recurrence and died of a second primary tumor 25 months later. One patient (No. 26) was never free of tumor and died of lymph node metastases 2 months after radiotherapy. The third patient (No. 27) died 6 months after brachytherapy, of his second recurrence. Thus, only 1 of the 12 T2 patients died of his primary tumor. Six (50%) suffered from a second primary cancer. Regional and distant recurrences were not found.

Two (Nos. 28, 29) of 9 patients (22%) with T3 tumors suffered from local recurrence. Three (33%) patients developed second primaries. One patient (11%) developed a pulmonary metastasis, and 1 (11%) developed a regional recurrence.

Five (63%) of 8 patients with T4 tumors suffered from local recurrence. One patient (12.5%) developed a second primary tumor. One patient (12.5%) developed lymph node metastasis, and 2 (25%), distant metastasis.

Neck Disease and Distant Metastasis. Four patients developed recurrence at the neck or were never free of lymph node metastasis. Distant metastasis was found in 3 patients and was located in the lung in all of them and additionally in the brain in 2.

Second Primary Cancer. The diagnosis of a secondary malignancy was obtained 13 times in 12 patients (35%) at the following locations: lung (6 patients), oropharynx (1), tongue (1), esophagus (1), prostate (1), bladder (1), and submandibular gland (adenocarcinoma; 1). The last patient also suffered from a third malignancy, a squamous cell carcinoma of the palate. He is now 47 months free of disease.

Follow-up. The cause of death was unrelated to

TABLE 4. OUTCOME OF ALL PATIENTS ACCORDING TO TUMOR STAGE

			Dead, Not Tumor-Related	*Dead of Second Primary*	*Dead, Tumor-Related*				*Alive With Tumor*
Stage	*Patients*	*Recurrence*			*T*	*N*	*M*	*Alive NED*	
I	2							2	
II	7	1	1	2				4	
III	13	2	4	1	2			6	
IV	12	7	3		2	3	1	2	1
	34	10	8	3	4	3	1	14	1

NED — no evidence of disease.

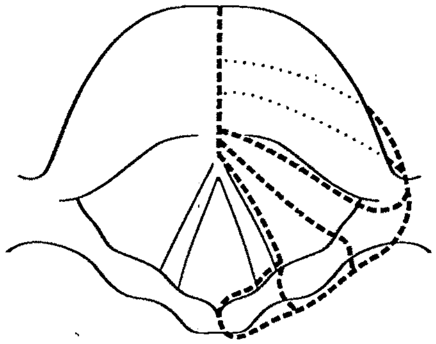

Fig 2. Laser resection lines in advanced carcinomas of supraglottis. Epiglottis has to be split in midline. Depending on extent of tumor, ventricular fold and aryepiglottic fold region are included in resection.

the tumor in 8 patients (Tables 4 and 5). Three patients died of a second primary cancer, 4 of a recurrent primary, 3 of lymph node metastasis, and 1 of distant metastasis. One patient is alive with disease 39 months after diagnosis. Another patient is alive and without evidence of locoregional recurrence but with pulmonary and brain metastasis. The survival of all patients who did not suffer from recurrence is shown in Fig 4. The 3-year overall survival is 62%, and the tumor-related survival 80%. Patients with T1 and T2 tumors have a significantly ($p = .0159$) better overall survival than patients with T3 and T4 tumors (Fig 5). The prognosis of the N0 and N1 cases is significantly ($p = .02641$) better than the one of the N2 and N3 cases (Fig 6). The analysis with regard to tumor stages revealed a significantly ($p = .0216$) better survival rate for the early stages, I and II, than for stages III and IV (5 years overall survival, 77% versus 39%).

Local and Regional Failure in Relation to Adjuvant Radiotherapy. There was no obvious difference between the patients with and without adjuvant radiotherapy (Table 6). This result is not unexpected, because most of the patients with radiotherapy belong to a group at higher risk than the patients without radiotherapy. The 2 patients (Nos. 6 and 7) who were irradiated because of positive margins remained free of local and regional recurrence.

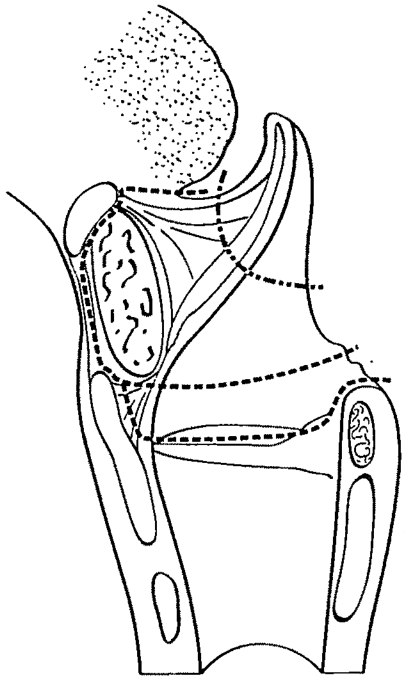

Fig 3. Laser resection lines (sagittal view). Upper line marks resection of suprahyoid tumor; others include preepiglottic space in case of infrahyoid tumors and tumors of ventricular fold.

Functional Outcome. The rate of functional problems as observed after conventional supraglottic partial resection is greatly decreased after transoral laser surgery. This is mainly reflected by the low number of only 6 tracheostomies after endoscopic procedures. In 1 patient (No. 33) in poor medical condition, a tracheostomy had to be performed 5 days postoperatively due to pneumonia. A nasogastric tube was placed in 26 patients. It was removed within 7 days in 14 patients and within 22 days in 7 more patients. In 5 patients, it was necessary to continue nasogastric tube feedings for more than 3 weeks. Two patients had received a tracheostomy years earlier for other reasons and kept it postoperatively. However, the patient with the postdiphtheric laryngeal stenosis complained of increased dysphagia after the laser surgical tumor resection and therefore underwent laryngectomy 16 months later.

Complications. Major hemorrhage occurred intraoperatively in 1 case and postoperatively in 2. All were treated by electrocautery. One patient in poor medical condition (No. 33) postoperatively suffered of pneumonia.

TABLE 5. OUTCOME OF ALL PATIENTS ACCORDING TO T CLASSIFICATION

					Dead, Tumor-Related				
T	*Patients*	*Recurrence*	*Dead, Not Tumor-Related*	*Dead of Second Primary*	*T*	*N*	*M*	*Alive NED*	*Alive With Tumor*
1	5		2					3	
2	12	3	1	2	1	1		7	
3	9	2	3	1	2			3	
4	8	5	2		1	2	1	1	1
	34	10	8	3	4	3	1	14	1

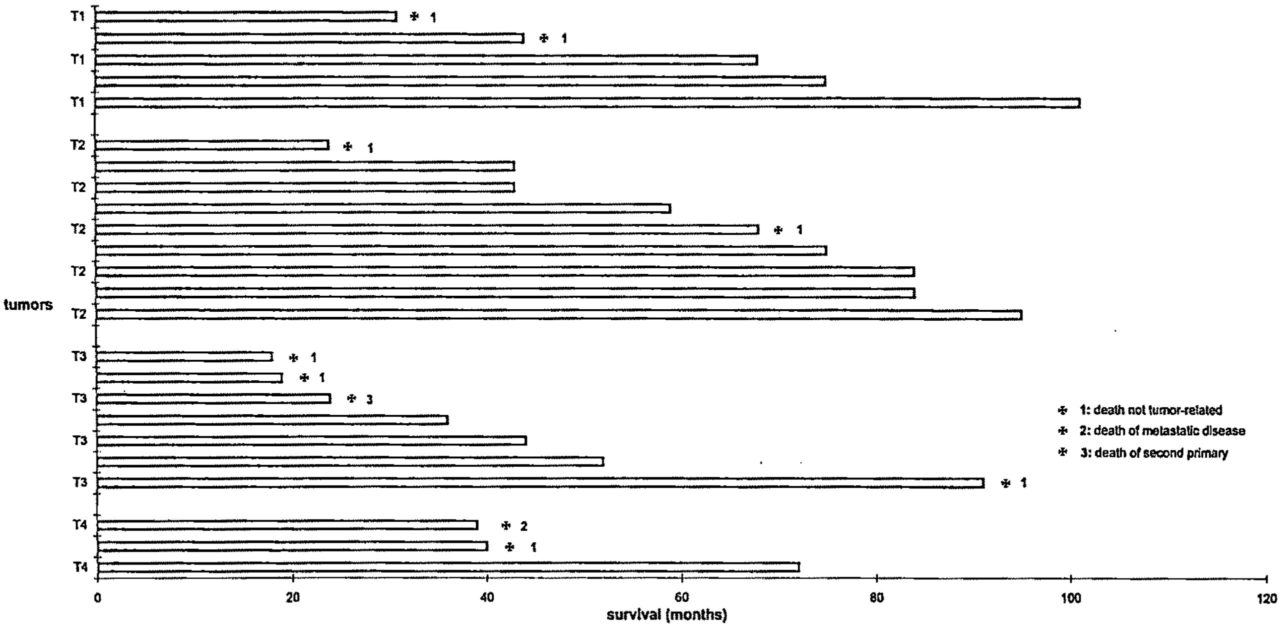

Fig 4. Survival times of 24 patients without local recurrence.

DISCUSSION

Transoral laser surgical horizontal partial resection of supraglottic carcinomas is a therapeutic modality that has yet to find its position in the treatment of cancer of the supraglottic larynx. Reports of success for this technique were published by Davis et al[9-11] and Zeitels et al[12-14] in the United States and by Steiner et al,[15-17] Eckel et al,[18,19] Rudert,[4,7] and Rudert and Werner[8,20] in Europe. The superior functional results of the transoral method are undoubtedly the lower morbidity and the reduced tendency toward aspiration. Chronic aspiration is observed in 40% to 70%[21] of patients following conventional open partial resection. This rate also depends on the age of the patients. In our patient population, only 6 patients required tracheostomy, and a 76-year-old patient was not decannulated at his own request. We no longer respect an age limit for this procedure, unless advanced age is associated with poor medical condition.

The comparison of the oncological outcome of our patients with the results of other authors is difficult, due to the difference in the patient population and in the tumor stages. Our patient population included a high number (50%) of advanced T3 and T4 tumors,

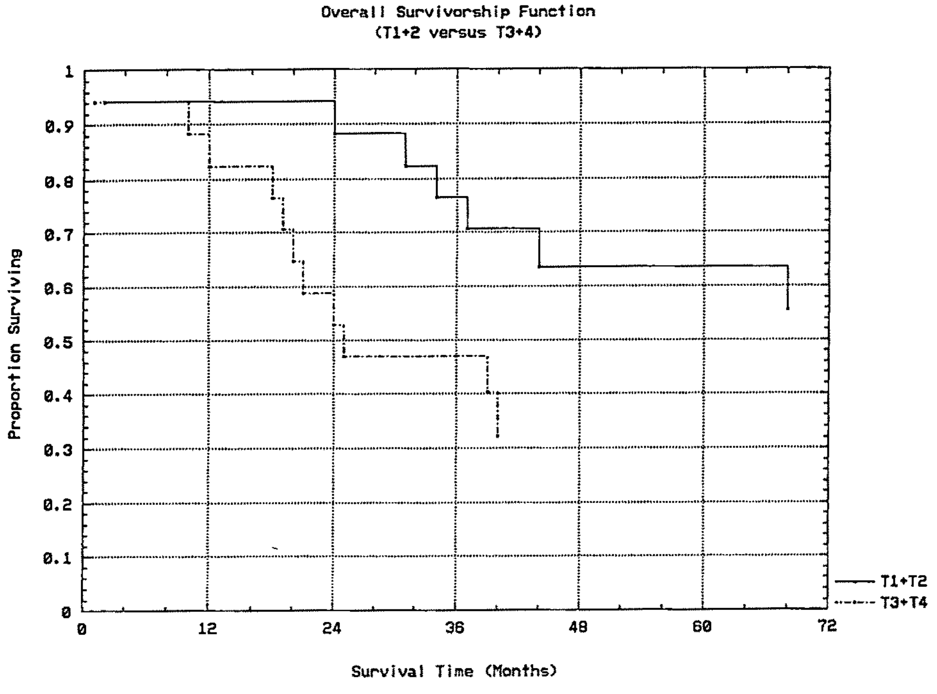

Fig 5. Overall survival of patients with T1 and T2 tumors versus T3 and T4 tumors ($p = .0159$).

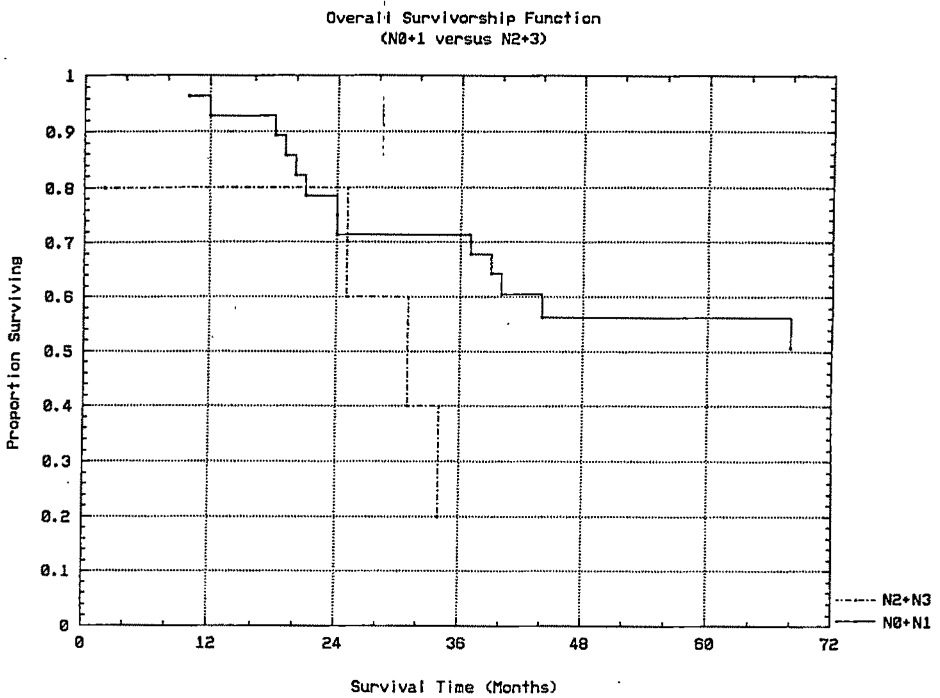

Fig 6. Overall survival of patients with N0 and N1 cases versus N2 and N3 cases (p = .02641).

and 12 cases were initially treated palliatively. Taking this into consideration, a tumor-related 3-year survival of 80% for the entire patient population is an acceptable result. Patients with T1 and T2 carcinomas have a significantly better prognosis than patients with T3 and T4 tumors (Fig 5). Analysis with regard to lymph node metastasis revealed that patients with N2 and N3 disease in comparison to patients with N0 and N1 disease have a very low chance for cure (Fig 6). Our current therapy includes an elective or curative neck dissection for all T2, T3, and T4 tumors. The analysis of tumor stages (Table 4) demonstrated best results for the 9 patients in stage I or II. Local recurrence was only found in 1 patient (No. 25) and was treated successfully by another laser surgical procedure. Of this group, no patient died of the tumor. Eckel[19] and Ambrosch et al[22] recently published comparable results. These results are also comparable to the outcome of open partial resections.[2,23-25] The outcome of the 25 patients with stage III or IV disease was significantly worse than for the stage I and II group. Regional or distant metastasis or second carcinomas were found in 56%. In this group, only patients with N0 or N1 neck disease had a solid chance for cure.

TABLE 6. LOCAL AND REGIONAL FAILURE IN RELATION TO ADJUVANT RADIOTHERAPY

Adjuvant Radiotherapy	*No Local or Regional Recurrence*	*Local Recurrence*	*Regional Recurrence*	*Local and Regional Recurrence*	*Total*
Yes	16	6	1	1	24
No	7	2		1	10
	23	8	1	2	34

Twelve of 25 patients with stage III or IV disease were treated palliatively with the laser, due to poor medical condition or advanced age, to avoid tracheostomy, or for refusal of laryngectomy (6 patients). Our analysis shows that it is these patients in particular who benefited from the transoral laser surgical technique. Some of them survived for a considerable time without local recurrence and without tracheostomy or problems with aspiration or swallowing and kept their voice. In earlier years these patients would have been treated by tracheostomy and possibly palliative radiotherapy. The decision for the organ-preserving transoral technique in selected advanced but technically laser surgical–resectable carcinomas is supported by the analysis of the complete follow-up of all patients, even though in early stages, radiotherapy might produce a comparable result.[26,27] The evaluation shows that only 4 of all 34 patients died of the local recurrence itself. This outcome in particular allows us to choose the surgical method that has the lowest morbidity and minimizes functional problems, ie, the transoral laser surgical method.

The transoral laser resection of these carcinomas requires certain techniques that seem to contradict classic rules of oncological surgery but are possible due to the cutting characteristics of the CO2 laser. Advanced tumors have to be divided and removed in 2 or more pieces. It is possible to do this relatively safely because of the hemostatic effect of the laser, which allows for the detection of the border between tumor and healthy tissue with the operating microscope. The resection has to include the preepiglottic space if the infrahyoid epiglottis is involved, and the preepiglottic space plus the paraglottic space if the false vocal cord is involved. Studies of Kirchner and Carter,[28] Sato et al,[29] and Reidenbach[30,31] have proven fibroelastic membranes that limit the central preepiglottic space and pose resistance to the tumor growth. Also, tumors growing through foramina of the epiglottic cartilage are enclosed by some sort of pseudocapsule.[32] The lateral region of the preepiglottic space, on the other hand, lacks complete anatomic borders. Tumors in this location encircle the dorsal end of the ventricle and from there grow into the paraglottic space and into the muscles of the vocal cord.[32] One has to agree with Kirchner[32] that T3 tumors of the described category have to be approached differently than T3 tumors of the epiglottis growing into the central preepiglottic space. The latter are suitable for a supraglottic partial resection, whereas the former are not, or are only rarely.

CONCLUSIONS

Carcinoma of the categories T1 and T2, as well as T3 tumors growing into the central portion of the preepiglottic space, are suitable for transoral laser surgical resection as long as adequate resection techniques are applied. It is mandatory that the lymphatic system of the neck be included in the therapeutic regimen in the same way as in open partial resection. In N0 cases, an elective lateral neck dissection must be applied, and in N+ cases, a curative neck dissection (usually a modified radical neck dissection) is necessary. In midline cases, the neck dissections are performed bilaterally. In pN+ cases and in cases in which the specimen of the primary tumor has positive margins, postoperative radiotherapy should be applied in addition.

The poor prognosis of advanced supraglottic carcinomas is much more influenced by metastasis and second primary cancer than by local recurrences. The transoral resection technique could therefore be an alternative to tracheostomy and palliative radiotherapy, in particular in patients not eligible for laryngectomy due to advanced tumor stage. The same is true for patients suffering from advanced age and poor medical condition, and for patients who refuse a laryngectomy. Palliative laser surgical resection can be performed also on patients with tumors blocking the airways, to avoid emergency tracheostomies or emergency laryngectomy. In these cases, laryngectomies can be performed without impairment by a tracheostoma.

REFERENCES

1. Alonso JM. Conservative surgery of cancer of the larynx. Trans Am Acad Ophthalmol Otolaryngol 1947;51:633-42.
2. Myers EN, Alvi A. Management of carcinoma of the supraglottic larynx: evolution, current concepts, and future trends. Laryngoscope 1996;106:559-67.
3. Rudert H. Laser surgery for carcinomas of the larynx and hypopharynx. In: Naumann HH, Panje WR, Herberhold C, eds. Head and neck surgery. Vol 3. Stuttgart, Germany: Thieme, 1998:355-70.
4. Rudert HH. Laser surgery in the management of carcinoma of the supraglottic larynx and hypopharynx. In: Myers EN, Bluestone CD, Brackmann DE, Krause CJ, eds. Advances in otolaryngology–head and neck surgery. Vol 12. St Louis, Mo: Mosby, 1998:61-80.
5. Rudert H. Instrumentarium für die CO2-Laser-Chirurgie. HNO 1989;37:76-7.
6. Rudert H, Werner JA. Partial endoscopic resections with CO2 laser in laryngeal cancer. I. Resection techniques. Laryngorhinootologie 1994;73:71-7.
7. Rudert HH. Transoral CO2-laser surgery in advanced supraglottic cancer. In: Smee R, Bridger GP, eds. Laryngeal cancer. Amsterdam, the Netherlands: Elsevier, 1994:457-61.
8. Rudert HH, Werner JA. Endoscopic resections of glottic and supraglottic carcinomas with the CO2 laser. Eur Arch Otorhinolaryngol 1995;252:146-8.
9. Davis RK, Shapshay SM, Strong SM. Transoral partial supraglottic resection using the CO2 laser. Laryngoscope 1983; 93:429-32.
10. Davis RK, Kelly SM, Hayes J. Endoscopic CO2 laser excisional biopsy of early supraglottic cancer. Laryngoscope 1991;101:680-3.
11. Davis RK, Hayes JK. Management of supraglottic cancer: selected endoscopic laser resection and postoperative irradiation. Adv Otorhinolaryngol 1995;49:231-6.
12. Zeitels SM, Vaughan CW, Domanowski GF. Endoscopic management of early supraglottic cancer. Ann Otol Rhinol Laryngol 1990;99:951-6.
13. Zeitels SM, Koufman JA, Davis RK, Vaughan CW. Endoscopic treatment of supraglottic and hypopharynx cancer. Laryngoscope 1994;104:71-8.
14. Zeitels SM, Davis RK. Endoscopic laser management of supraglottic cancer. Am J Otolaryngol 1995;16:2-11.
15. Steiner W. Results of curative laser microsurgery of laryngeal carcinomas. Am J Otolaryngol 1993;14:116-21.
16. Steiner W, Ambrosch P. CO2 laser microsurgery for laryngeal cancer. In: Smee R, Bridger GP, eds. Laryngeal cancer. Amsterdam, the Netherlands: Elsevier, 1994:369-72.
17. Steiner W. Experiences in endoscopic laser surgery of malignant tumors of the upper aero-digestive tract. Adv Otorhinolaryngol 1988;39:135-44.
18. Eckel HE, Thumfart WF. Laser surgery for the treatment of larynx carcinomas: indications, techniques, and preliminary results. Ann Otol Rhinol Laryngol 1992;101:113-8.
19. Eckel HE. Endoscopic laser resection of supraglottic carcinoma. Otolaryngol Head Neck Surg 1998;117:681-7.
20. Rudert H, Werner JA. Endoskopische Teilresektionen mit dem CO2-Laser bei Larynxcarcinomen. II. Ergebnisse. Laryngorhinootologie 1995;74:294-9.
21. Suarez C, Rodrigo JP, Herranz J, Diaz C, Fernandez JA.

Complications of supraglottic laryngectomy for carcinomas of the supraglottis and the base of the tongue. Clin Otolaryngol 1996;21:87-90.

22. Ambrosch P, Kron M, Steiner W. Carbon dioxide laser microsurgery for early supraglottic carcinoma. Ann Otol Rhinol Laryngol 1998;107:680-8.

23. DeSanto LW. Early supraglottic cancer. Ann Otol Rhinol Laryngol 1990;99:593-7.

24. Bocca E. Surgical management of supraglottic cancer and its lymph node metastases in a conservative perspective. Ann Otol Rhinol Laryngol 1991;100:261-7.

25. Herranz-González J, Gavilán J, Martínez-Vidal J, Gavilán C. Supraglottic laryngectomy: functional and oncologic results. Ann Otol Rhinol Laryngol 1996;105:18-22.

26. Mendenhall WM, Parsons JT, Stringer SP, Cassisi NJ, Million RR. Carcinoma of the supraglottic larynx: a basis for comparing the results of radiotherapy and surgery. Head Neck 1990;12:204-9.

27. Santos CR, Kowalski LP, Magrin J, et al. Prognostic factors in supraglottic carcinoma patients treated by surgery or radiotherapy. Ann Otol Rhinol Laryngol 1998;107:697-702.

28. Kirchner JA, Carter D. Intralaryngeal barriers to the spread of cancer. Acta Otolaryngol (Stockh) 1987;103:503-13.

29. Sato K, Kurita S, Hirano M. Location of the preepiglottic space and its relationship to the paraglottic space. Ann Otol Rhinol Laryngol 1993;102:930-4.

30. Reidenbach MM. The periepiglottic space: topographic relations and histological organisation. J Anat 1996;188:173-82.

31. Reidenbach MM. Borders and topographic relationships of the paraglottic space. Eur Arch Otorhinolaryngol 1997;254:193-5.

32. Kirchner JA. Glottic-supraglottic barrier: fact or fantasy? Ann Otol Rhinol Laryngol 1997;106:700-4.

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MANAGEMENT OF THE NECK IN CANCER OF THE LARYNX

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In the Department of Otolaryngology at the University of Pittsburgh School of Medicine, cancer of the larynx is usually treated by primary surgery. Radiotherapy is used as adjuvant treatment in certain patients who have cancer that has adverse histologic features such as perineural, vascular, and/or cartilage invasion. With this approach, patients rarely develop local recurrence. Patient survival is therefore unlikely to be improved by changes to the management of the primary tumor. Survival may, however, be improved by reducing the incidence of recurrence in the neck, as well as distant. Hence, we have adopted an aggressive surgical approach to the cN+ as well as the N0 neck. The theoretical basis for this aggressive surgical approach to the neck will be considered under the following headings: staging, regional control, distant metastasis, survival, choice of neck dissection, and the pathologically positive elective neck dissection.

KEY WORDS — head and neck neoplasms, laryngeal neoplasms, lymphatic metastasis, neck dissection, squamous cell carcinoma, staging.

STAGING

It is reasonable to assume that occult nodal metastasis will become clinically apparent if left untreated, as the incidence of histologically positive nodes in electively treated necks corresponds with the nodal conversion rate of untreated N0 necks.[1] Understaging, and consequently undertreatment, of the occult positive neck is therefore likely to adversely affect regional control and survival. The central issue pertaining to the various staging modalities is the sensitivity, or false-negative rate, in the N0 neck. Specificity only assumes importance when the treatment of the false-positive neck has significant associated morbidity.

Twelve percent of tumor-positive neck dissections contain only micrometastases[2]; of clinically N0 necks with occult metastases, 25% contain only micrometastases.[3] Current preoperative staging modalities for the N0 neck are therefore unable to exceed a sensitivity of 75% without sacrificing specificity.[3]

Computed tomography (CT) scanning, magnetic resonance imaging (MRI), and ultrasound rely principally on nodal size as a criterion for malignancy. There is, however, a trade-off between sensitivity and specificity when nodal diameter is used to stage the N0 neck. The smaller the minimum nodal diameter used as a criterion for malignancy, the greater the sensitivity, but the lower the specificity, and vice versa. Most authorities recommend a minimum nodal size criterion for malignancy of 10 mm.[4] Yet the great majority of lymph node metastases in the N0 neck are smaller than 10 mm in diameter,[4] and nonmalignant nodes may vary in size between 2 mm and 2 cm.[5] Although 74% of lymph node metastases have central necrosis, this percentage is much reduced with smaller nodes found in the N0 neck.[4]

Ultrasound-guided fine needle aspiration cytology (USGFNAC) has overcome the problems relating to specificity associated with CT, MRI, and ultrasound. It has a reported specificity approaching 100%.[6] Yet, the sensitivity of USGFNAC in the N0 neck ranges between 44% and 73%.[3] Van den Brekel et al[7] reported an 18% failure rate in the neck, with a 71% salvage rate, for early oral cavity carcinomas initially staged and followed by USGFNAC. Elective neck dissection might well have avoided these failures, and improved overall outcome.

Extracapsular spread (ECS) is an important prognostic indicator of regional and distant failure and survival.[8-11] It is important to identify patients with ECS, as they appear to benefit from adjuvant irradiation and chemotherapy.[1,12-16] Extracapsular spread occurs in 23% of metastatic nodes <10 mm in diameter.[8] Were reliance placed on imaging alone to stage the N0 neck, then a significant number of patients with ECS would remain untreated until cervical metastasis becomes clinically apparent.

Because of these limitations of imaging, we advocate the use of elective neck dissection in combination with intraoperative and pathological evaluation

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to stage and plan the treatment of the N0 neck.

Intraoperative diagnosis of occult metastasis in the clinically N0 neck has a sensitivity of 44% and a specificity of 63% when reliance is placed on the surgeon's ability to evaluate lymph nodes by inspection and palpation only. Frozen section diagnosis of malignancy has a sensitivity and specificity of 100% per suspicious node. In N0 necks judged by the surgeon to harbor occult metastasis, frozen section has a sensitivity of 71%, a specificity of 100%, and an accuracy of 92.3% per neck.[17] It has been our practice to sample suspicious nodes discovered at the time of elective neck dissection, and to submit the nodes for frozen section. If metastasis is diagnosed by frozen section, then the staging neck dissection is converted to a therapeutic neck dissection, usually modified radical neck dissection type 1.

Surgical staging therefore has a number of benefits. The risk of understaging the N0 neck is minimized. Patients with ECS, who are at high risk for recurrent cancer and often benefit from adjuvant irradiation and chemotherapy, can be identified. The use of chemoradiation therapy is reserved for the ECS group, and is therefore used sparingly, appropriately, and effectively. Patients with more than 2 nodes without ECS are also offered adjunctive radiotherapy.

REGIONAL CONTROL

There are a number of reports of significant improvement in regional control if the N0 neck is treated by elective neck dissection with or without adjuvant irradiation, as opposed to watchful waiting.[18-22]

It has been proposed that elective neck dissection be performed only if the risk of occult metastasis exceeds 15% to 30%.[1,5,22-24] Corrected for pathological understaging (7.5%),[25] 23% to 38% of patients treated by watchful waiting might ultimately fail in the neck if such a management policy were to be followed. Only 11% to 56% of patients managed by watchful waiting and that subsequently fail in the neck are successfully salvaged.[24,26-28] We therefore consider the above-mentioned thresholds for elective neck dissection to be unacceptably high.

Extracapsular spread is associated with increased risk of regional failure.[9,29] The incidence of ECS correlates with nodal size, and occurs with 23% of nodes <1 cm, 44% of nodes 1 to 2 cm, 53% of nodes 2 to 3 cm, and 74% of nodes >3 cm.[9] Small metastatic nodes without ECS, if left untreated, will increase in size and may subsequently manifest ECS. By intervening early in the course of the disease, elective neck dissection may theoretically prevent this progression to ECS. Adjuvant radiotherapy, and possibly chemotherapy, improves regional control in patients with ECS.[1,12,14-16] Elective neck dissection permits earlier detection of patients with ECS who may benefit from adjuvant therapy.

Said somewhat differently, while watchful waiting will detect many patients who are operable, some will present with inoperable neck disease.[30] Many of the operable group will be incurable because of extensive ECS at presentation. Not doing an elective neck dissection therefore may deprive some of these patients of the opportunity to be cured.

DISTANT METASTASIS

The incidence of distant metastasis is related to tumor burden in the neck,[1,8,31,32] the duration of nodal metastasis,[33] the number[8] and level[2,8,31] of nodal metastases, and the presence of ECS.[8,34] Metastasis to lymph nodes generally first involves the primary and secondary echelons, and then spreads down the neck toward the superior mediastinum.[2] Elective neck dissection should reduce the risk of distant metastasis by minimizing tumor burden, restricting the duration of metastasis, and reducing the number of nodes, and has been shown to reduce the likelihood of involvement of the lower levels of the neck.[20]

We reported the use of chemoradiation in patients with ECS. Despite the nonrandomized nature of the study, it would appear that chemoradiation did improve patient outcome.[16] Elective neck dissection permits the early identification of such patients with ECS who may benefit from chemoradiation.

SURVIVAL

From the preceding discussion, it is reasonable to assume that an aggressive surgical approach to the N0 neck should not only increase the disease-free interval, but also improve disease-specific survival.

Two prospective randomized studies of elective neck dissection versus watchful waiting in N0 squamous carcinoma of the head and neck reported no significant difference in survival.[35,36] Neither study, however, had adequate power to detect a significant difference in outcome.[33,37] In the study by Vandenbrouck et al,[36] 45% of deaths were unrelated to regional failure. The study by Fakih et al[35] did not control for ECS, tumor margins, or contralateral regional failure, and median follow-up was only 20 months. Although the survival benefit of elective neck dissection, as opposed to watchful waiting, for head and neck squamous carcinoma has not been proven in a properly designed study, Lydiatt et al[38] reported a survival benefit for elective neck dissection in a nonrandomized study of carcinoma of the oral tongue.

TABLE 1. INCIDENCE OF OCCULT IPSILATERAL NODAL METASTASIS ACCORDING TO TUMOR SITE

	UPMC Studies	*Other Studies*
Glottic (T3/4)	9% to 10.4%[34,36]	3% to 36%[13,22,39]
Transglottic (FVC and glottis)	12%[46]	11%[22,47]
Supraglottic	41%[15]	16% to 43%[13,22,39,42,48,49]
Subglottic		65%[50]
Pyriform fossa		38% to 56%[13,22,51]

UPMC — University of Pittsburgh Medical Center, FVC — false vocal cord.

CHOICE OF NECK DISSECTION

The decision whether to perform selective or comprehensive neck dissection, and whether to perform ipsilateral or bilateral neck dissection, is determined principally by tumor site, extent, and intraoperative staging.

Laryngeal carcinoma rarely metastasizes to level I[13,39-42] or level V.[39,42-44] Selective neck dissection, with sparing of levels I and V, is therefore an appropriate staging and therapeutic measure if performed when occult metastasis, if at all present, is likely to be confined to the first-echelon nodes. Should there be evidence of nodal metastasis at the time of selective neck dissection, then there is an increased likelihood of involvement of levels I[42,45] and V,[39,42,43,45] and a comprehensive neck dissection should be performed.

Table 1[13,15,22,34,36,39,42,46-51] summarizes the reported incidence of occult ipsilateral lymph node metastasis in accordance with the location of the laryngeal tumor. On the basis of these data, we recommend elective ipsilateral selective neck dissection (levels II through IV) with supraglottic, transglottic, and advanced glottic carcinoma. Level VI (including thyroid lobectomy) is routinely dissected with glottic and subglottic carcinoma.

The subglottis drains principally to the paratracheal, pretracheal, and prelaryngeal lymphatics.[50,52] Carcinoma of the subglottis has a 65% incidence of occult paratracheal nodal metastasis,[50] and may involve the thyroid gland by direct extension and/or by lymphatic spread.[52] Inadequate treatment of the paratracheal lymphatics and the thyroid gland is the principal reason for the association of subglottic carcinoma with peristomal recurrence.[53-55] It has therefore been proposed that management of carcinoma of the subglottis should include dissection of level VI, a total thyroidectomy, and adjuvant radiotherapy to the lower neck and superior mediastinum.[52,53,55]

TABLE 2. INCIDENCE OF OCCULT CONTRALATERAL NODAL METASTASIS ACCORDING TO TUMOR SITE

	UPMC Studies	*Other Studies*
Glottic (T3/4)		1% to 2%[27,56]
Transglottic (FVC and glottis)		5%[27]
Supraglottic	17%[57]	13% to 30%[27,49]
Medial wall of pyriform fossa	14%[19]	
Lateral wall of pyriform fossa	5%[19]	

TABLE 3. CHOICE OF ELECTIVE NECK DISSECTION

	Ipsilateral Neck	*Contralateral Neck*
Glottic (T3/4)	II-IV, VI	
Transglottic (FVC and glottis)	II-IV, VI	
Supraglottic	II-IV	II-IV
Subglottic	II-IV, VI	VI
Medial wall of pyriform fossa	II-IV, VI	II-IV
Intraoperative N+ staging	MND/RND	MND/RND

MND — modified neck dissection, RND — radical neck dissection.

Elective dissection of the contralateral neck is governed by tumor site (Table 2[19,27,49,56,57]). Carcinoma of the glottis[27,56] uncommonly metastasizes to the contralateral neck, and does not require contralateral neck dissection. An exception to this policy is tumor extension through the paraglottic space to involve the medial wall of the pyriform fossa, as tumors involving the medial wall of the pyriform fossa have a propensity for bilateral lymphatic metastasis.[19] Tumors involving the supraglottis similarly require bilateral elective neck dissections[27,57] (levels II through IV; Table 3).

PATHOLOGICALLY POSITIVE ELECTIVE NECK DISSECTIONS

When nodal metastasis is detected on routine pathological examination of a selective neck dissection specimen, the surgeon has the following therapeutic options: 1) completion neck dissection, 2) adjuvant irradiation and/or chemotherapy, and 3) watchful waiting.

The success of salvage neck dissection depends on the mobility of the nodal recurrences. Surgical salvage is therefore rarely successful with regional failure following radical neck dissection.[9,58] Good salvage rates can, however, be expected following neck dissection for recurrences within levels not previously dissected.[58]

Factors that may influence the indications for and choice of additional therapy include the number and distribution of nodal metastases, ECS, and the need for adjuvant therapy to the primary tumor bed. We have previously reported that the presence or absence of nodal metastasis, in the absence of ECS, does not affect the outcome of patients treated with neck dissection.[10,29] We generally would not give adjuvant therapy to patients who have occult metastasis in an elective neck dissection specimen, unless there is extensive nodal metastasis approaching the perimeter of the dissection, or ECS, or if the patient requires adjuvant radiotherapy for the laryngeal primary tumor.

CONCLUSIONS

An aggressive surgical approach to the neck in laryngeal carcinoma has a number of potential benefits. It ensures accurate staging and improves regional control. By improving regional control and by permitting early identification of patients who may benefit from adjuvant therapy, this approach should reduce the incidence of distant metastasis and improve survival.

REFERENCES

1. Snow GB, Patel P, Leemans CR, Tiwari R. Management of cervical lymph nodes in patients with head and neck cancer. Eur Arch Otorhinolaryngol 1992;249:187-94.

2. van den Brekel MWM, Snow GB. Assessment of lymph node metastases in the neck. Eur J Cancer B Oral Oncol 1994; 30B:88-92.

3. van den Brekel MWM, van der Waal I, Meyer CJLM, Freeman JL, Castelijns JL, Snow GB. The incidence of micrometastases in neck dissection specimens obtained from elective neck dissections. Laryngoscope 1996;106:987-91.

4. van den Brekel MWM, Castelijns JA, Snow GB. Detection of lymph node metastases in the neck: radiologic criteria. Radiology 1994;192:617-8.

5. Ali S, Tiwari RM, Snow GB. False positive and false negative neck nodes. Head Neck Surg 1985;8:78-82.

6. van den Brekel MWM, Stell HV, Castelijns JA, Croll GA, Snow GB. Lymph node staging in patients with clinically negative neck examinations by ultrasound and ultrasound-guided fine needle aspiration cytology. Am J Surg 1991;162:362-6.

7. van den Brekel MWM, Castelijns JA, Snow GB. Diagnostic evaluation of the neck. Otolaryngol Clin North Am 1998; 31:601-20.

8. Leemans CR, Tiwari R, Nauta JJP, van der Waal I, Snow GB. Regional lymph node involvement and its significance in the development of distant metastases in head and neck carcinoma. Cancer 1993;71:452-6.

9. Snow GB, Annyas AA, Van Slooten EA, Bartelink H, Hart AA. Prognostic factors of neck node metastasis. Clin Otolaryngol 1982;7:185-92.

10. Johnson JT, Myers EN, Bedetti CD, Barnes EL, Schramm VL, Thearle PB. Cervical lymph node metastases: incidence and implications of extracapsular carcinoma. Arch Otolaryngol 1985;111:534-7.

11. Hirayabashi H, Koshii K, Uno K, et al. Extracapsular spread of squamous carcinoma in neck lymph nodes: prognostic factor of laryngeal cancer. Laryngoscope 1991;101:502-6.

12. Annyas AA, Snow GB, van Slooten EA, et al. Prognostic factors in neck node metastasis: their impact on planning a treatment regime. Read before the American Society of Head and Neck Surgeons, Los Angeles, April 6, 1979.

13. Byers RM. Modified neck dissection. A study of 967 cases from 1970-1980. Am J Surg 1985;150:414-21.

14. Byers RM, Wolf PF, Ballantyne AJ. Rationale for elective modified neck dissection. Head Neck 1988;10:160-7.

15. Snyderman NL, Johnson JT, Schramm VL, Myers EN, Bedetti CD, Thearle P. Extracapsular spread of carcinoma in cervical lymph nodes: impact upon survival in patients with carcinoma of the supraglottic larynx. Cancer 1985;56:1597-9.

16. Johnson JT, Wagner RL, Myers EN. A long-term assessment of adjuvant chemotherapy on outcome of patients with extracapsular spread of cervical metastases from squamous carcinoma of the head and neck. Cancer 1996;77:181-5.

17. Rassekh CH, Johnson JT, Myers EN. Accuracy of intraoperative staging of the N0 neck in squamous cell carcinoma. Laryngoscope 1995;105:1334-6.

18. Cachin Y. Management of cervical nodes in head and neck cancer. In: Rhys Evans PH, Robin PE, Fielding JWL, eds. Head and neck cancer. London, England: Castle House Publications Ltd, 1983:168-77.

19. Johnson JT, Bacon GW, Myers EN, Wagner RL. Medial vs lateral wall pyriform sinus carcinoma. Head Neck 1994;16: 401-5.

20. Hughes CJ, Gallo O, Spiro RH, Shah JP. Management of occult metastases in oral cavity squamous cell carcinoma. Am J Surg 1993;166:380-3.

21. Weber PC, Johnson JT, Myers EN. The impact of bilateral neck dissection on pattern of recurrence and survival in supraglottic carcinoma. Arch Otolaryngol Head Neck Surg 1994;120: 703-6.

22. Ogura JH, Biller HF, Wette R. Elective neck dissection for pharyngeal and laryngeal cancers. An evaluation. Ann Otol Rhinol Laryngol 1971;80:646-53.

23. Bocca E, Pignataro O, Oldini C, Cappa MD. Functional neck dissection: an evaluation and review of 843 cases. Laryngoscope 1984;94:942-5.

24. Weiss MH, Harrison LB, Isaacs RS. Use of decision analysis in planning a management strategy for the stage N0 neck. Arch Otolaryngol Head Neck Surg 1994;120:699-702.

25. DeSanto LW, Holt JJ, Beahrs OH, O'Fallon WM. Neck dissection: is it worthwhile? Laryngoscope 1982;92:502-9.

26. Ho CM, Lam KH, Wei WI, Lau SK, Lam LK. Occult lymph node metastasis in small oral tongue cancers. Head Neck 1992;14:359-63.

27. Biller HF, Davis WH, Ogura JH. Delayed contralateral cervical metastasis with laryngeal and hypopharyngeal cancers. Laryngoscope 1971;81:1499-502.

28. Yuen AP, Ho CM, Wei WI, Lam LK. Prognosis of recurrent laryngeal carcinoma after laryngectomy. Head Neck 1995;

17:526-30.

29. Johnson JT, Barnes EL, Myers EN, Schramm VL, Borochovitz D, Sigler BA. The extracapsular spread of tumors in cervical node metastases. Arch Otolaryngol 1981;107:725-9.

30. Yuen AP, Wei WI, No CM. Results of surgical salvage for radiation failures of laryngeal carcinoma. Otolaryngol Head Neck Surg 1995;112:405-9.

31. Grandi C, Alloisio E, Moglia D, et al. Prognostic significance of lymphatic spread in head and neck carcinoma: therapeutic implications. Head Neck Surg 1985;8:67-73.

32. Ellis ER, Mendenhall WM, Rao PV, Parsons JT, Spangler AE, Million RR. Does node location affect the incidence of distant metastases in head and neck carcinoma? Int J Radiation Oncol Biol Phys 1989;17:293-7.

33. Steiner W, Hommerich CP. Diagnosis and treatment of the N0 neck of carcinomas of the upper aerodigestive tract. Report of an International Symposium, Gottingen, Germany, 1992. Eur Arch Otorhinolaryngol 1993;250:450-6.

34. Johnson JT, Myers EN, Hao S-P, Wagner RL. Outcome of open surgical therapy for glottic carcinoma. Ann Otol Rhinol Laryngol 1993;102:752-5.

35. Fakih AR, Rao RS, Borges AM, Patel AR. Elective vs therapeutic neck dissection in early carcinoma of the oral tongue. Am J Surg 1989;158:309-13.

36. Vandenbrouck C, Sancho-Garnier H, Chassagne D, Saravane D, Cachin Y, Micheau C. Elective versus therapeutic radical neck dissection in epidermoid carcinoma of the oral cavity. Cancer 1980;46:386-90.

37. Davidson J, Biem J, Detsky A. The clinically negative neck in patients with early oral cavity carcinoma: a decision-analysis approach to management. J Otolaryngol 1995;24:323-9.

38. Lydiatt DD, Robbins KT, Byers RM, Wolf PF. Treatment of stage I and II oral tongue cancer. Head Neck 1993;15:308-12.

39. Candela FC, Shah J, Jaques DP, Shah JP. Patterns of cervical node metastases from squamous carcinoma of the larynx. Arch Otolaryngol Head Neck Surg 1990;116:432-5.

40. Feldman DE, Applebaum EL. The submandibular triangle in radical neck dissection. Arch Otolaryngol 1977;103:705-6.

41. Lindberg R. Distribution of cervical nodal metastases from squamous cell carcinoma of the upper respiratory and digestive tracts. Cancer 1972;229:1446-9.

42. Shah JP. Patterns of cervical lymph node metastasis from squamous carcinoma of the upper aerodigestive tract. Am J Surg 1990;160:405-9.

43. Davidson BJ, Kulkarny V, Delacure MD, Shah JP. Posterior triangle metastases of squamous cell carcinoma of the upper aerodigestive tract. Am J Surg 1993;166:395-8.

44. Skolnik EM, Yee KF, Friedman M, Goldman TAI. The posterior triangle in radical neck surgery. Arch Otolaryngol 1976;102:1-4.

45. Kowalski LP, Franco EL, de Andrade Sobrinho J. Factors influencing regional lymph node metastasis from laryngeal carcinoma. Ann Otol Rhinol Laryngol 1995;104:442-7.

46. Hao S, Myers EN, Johnson JT. T3 glottic carcinoma revisited — transglottic vs pure glottic carcinoma. Arch Otolaryngol Head Neck Surg 1995;121:166-70.

47. Mittal B, Marks JE, Ogura JH. Transglottic carcinoma. Cancer 1984;53:151-61.

48. Ramadan HH, Allen GC. The influence of elective neck dissection on neck relapse in N0 supraglottic carcinoma. Am J Otolaryngol 1993;14:278-81.

49. Levendag P, Vikram B. The problem of neck relapse in early stage supraglottic cancer: results of different treatment modalities for the clinically negative neck. Int J Radiat Oncol Biol Phys 1987;46:1621-4.

50. Harrison DFN. The pathology and management of subglottic cancer. Ann Otol Rhinol Laryngol 1971;80:6-12.

51. Jones AS, Stell PM. Is laterality important in neck node metastases in head and neck cancer? Clin Otolaryngol 1991;16:261-5.

52. Fagan JJ, Kaye PV. Management of the thyroid gland with laryngectomy for cT3 glottic carcinoma. Clin Otolaryngol 1997;22:7-12.

53. Fagan JJ, Loock JW. Tracheostomy and peristomal recurrence. Clin Otolaryngol 1996;21:328-30.

54. Rubin J, Johnson JT, Myers EN. Stomal recurrence after laryngectomy: interrelated risk factor study. Otolaryngol Head Neck Surg 1990;103:805-12.

55. Harrison DFN. The thyroid gland in the management of laryngopharyngeal cancer. Arch Otolaryngol 1973;97:301-2.

56. Till JE, Bruce WR, Elwan A, et al. A preliminary analysis of the end results for cancer of the larynx. Laryngoscope 1975;85:259-75.

57. Lutz CK, Johnson JT, Wagner RL, Myers EN. Supraglottic carcinoma: patterns of recurrence. Ann Otol Rhinol Laryngol 1990;99:12-7.

58. Grandi C, Mingardo M, Guzzo M, Licitra L, Podrecca S, Molinari R. Prognostic significance of lymphatic spread in head and neck carcinoma: therapeutic implications. Head Neck Surg 1985;8:67-73.

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MINIMALLY INVASIVE DEVICE TO EFFECT VOCAL FOLD LATERALIZATION

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Despite many operative procedures focused on vocal fold lateralization, none has achieved an acceptable level of dependability. Bilateral vocal fold abductor paralysis is treated by arytenoidectomy, cordotomy, suture lateralization, or partial cordectomy. Tracheotomy remains the gold standard for maximizing the airway and preserving phonatory function. We have developed a device that is minimally invasive, tunable, and reversible, with the potential for lateralization or medialization of the vocal process. The device consists of a polyethylene collar, a Vitallium cam, and a double-helix core for engaging soft tissue. It is introduced through a circular opening in the thyroid cartilage by a modified thyroplasty approach. Both the first and second iterations of this device have been evaluated for clinical effectiveness in 9 sheep by means of photographic and video documentation. Effectiveness in humans is currently being assessed. The results of the animal study permit us to have substantial optimism with respect to the clinical application of this device.

KEY WORDS — bilateral abductor paralysis, lateralization.

PROBLEM

A patient with an inability to lateralize both vocal folds is in a precarious position with respect to the airway, even though the voice may be minimally impaired. Most patients who have irreversible abductor paralysis end up having some form of surgical treatment directed toward enhancement of the airway (often with the sacrifice of voicing).

The gold standard for airway protection is a tracheotomy; however, because of the nuisance and inconvenience involved with tracheotomy tube maintenance and tracheostoma care, other procedures, directed more specifically toward the endolarynx, are used relatively frequently.[1-11] Surgical approaches to the arytenoid and vocal fold include arytenoidectomy, done endoscopically or through an open approach, and partial vocal fold resection. Suture lateralization may be performed either openly or endoscopically. Often, a microscope is used to enhance visualization and the laser is used as an alternative surgical modality. All of these procedures create irreversible changes in the endolarynx and very frequently reduce the quality of the voice. The end results of the surgical procedure are never known until all of the tissue healing has taken place. Frequently, the lateralized vocal fold tends to be medialized again through contracture associated with the healing process.

DESCRIPTION OF DEVICE

The lateralization device consists of a polyethylene collar with medial and lateral flanges, an inner cam, and a double-helix screw designed to engage soft tissue. Additional instrumentation includes a circular punch, a collar forceps, and cam and piston drives.

DESCRIPTION OF PROCEDURE

Adult sheep were utilized in this study. All animals were sedated with an intramuscular combination of ketamine hydrochloride (22 mL/kg) and xylazine (0.2 mg/kg) and anesthetized with halothane-oxygen. Supplemental dosages were administered as needed to maintain an appropriate level of anesthesia.

Under general anesthesia, the anterior neck was shaved, prepared, and draped for sterile surgery with the head in a neutral position. A transverse incision was made at the mid–thyroid ala level. The strap muscles were incised and reflected laterally. The recurrent laryngeal nerve was identified on the right side, divided, and ligated with 3-0 silk sutures.

A rigid laryngoscope was inserted into the airway for direct visualization of the laryngeal structures. This maneuver was aided by a 4.0-mm 0° rigid endo-

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From the Departments of Otolaryngology–Head and Neck Surgery (Cummings, Redd, Flint) and Pathology (Westra), Johns Hopkins University, Baltimore, Maryland. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Johns Hopkins University.

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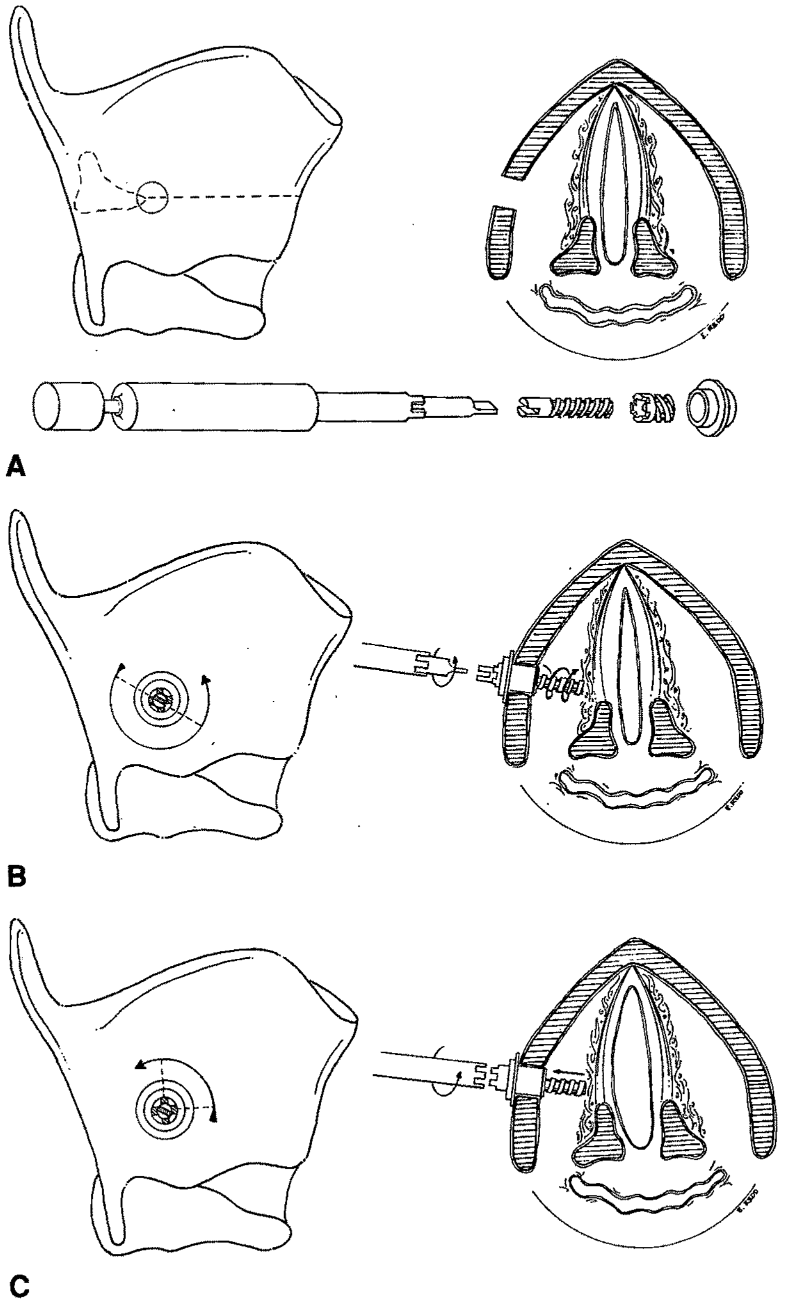

Fig 1. Diagrams of procedure. A) Center point of window is made at level of true vocal fold approximating vocal process. One-centimeter window is cut with circular punch, and thyroid cartilage is removed. B) Prosthesis snaps into window. Inner cam is advanced to vocalis process without penetrating endolarynx. C) Outer cam is backed out to lateralize engaging device and true vocal fold.

scope with video surveillance. The level of the true vocal fold was identified with a 22-gauge needle passed through the thyroid cartilage. A 1-cm-diameter circular window was then created in the thyroid cartilage to accommodate the prosthesis. The center point of the window was made at the level of the true vocal fold approximating the vocal process, determined during laryngoscopy. The window was cut with a 1-cm circular punch, and the thyroid cartilage was removed (Fig 1A). The flanged polyethylene collar was inserted such that the flange was adjacent to the outer thyroid lamina. The inner and outer cams were then placed within the collar and advanced medially until fully flush with the collar. The double-helix engaging device (inner cam) was advanced with a counterclockwise turn to grasp the thyroarytenoid tissue lateral to the vocalis process. With endoscopic control, the inner cam may be advanced to the vocalis process without penetrating the endolarynx (Fig 1B). The polyethylene collar was then grasped with the collar forceps while the outer cam was backed out, lateralizing the engaging device and thus the true vocal fold (Fig 1C). Vocal fold lateralization was documented endoscopically during this procedure. The wound was then irrigated and closed with interrupted 3-0 Vicryl sutures.

The sheep recovered for 1 week in the animal care facility and then were kept at the animal farm for 3 or 6 months. The animals were then submitted to vid-

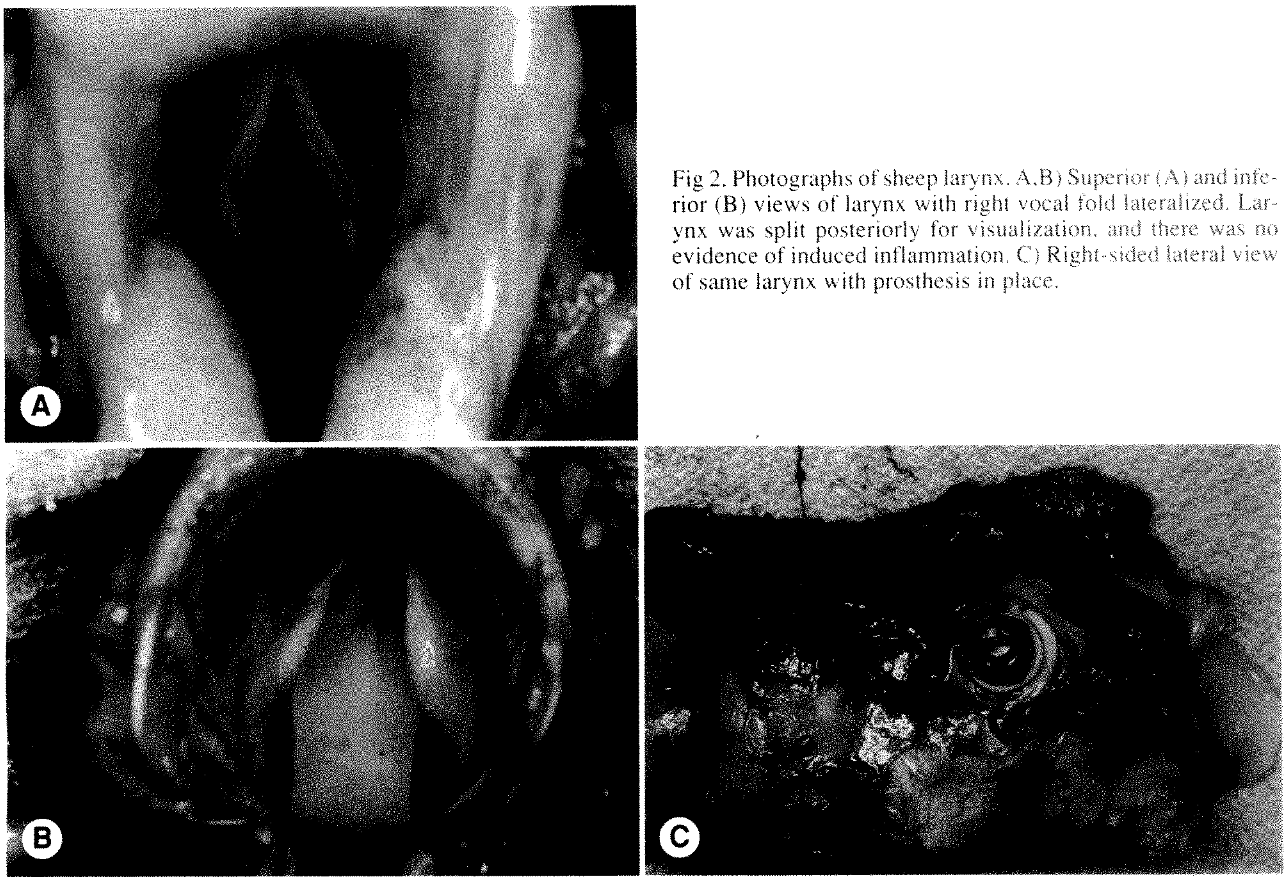

Fig 2. Photographs of sheep larynx. A,B) Superior (A) and inferior (B) views of larynx with right vocal fold lateralized. Larynx was split posteriorly for visualization, and there was no evidence of induced inflammation. C) Right-sided lateral view of same larynx with prosthesis in place.

eoscopy and euthanized. The larynges were harvested for histopathologic as well as gross anatomic interpretation. This study involved lateralization devices of 2 separate designs. Phase 1 incorporated a coil-like structure as the inner cam, which proved to be minimally effective. The phase 2 prostheses involved replacement of the coil with a double-helix screw, which proved to be uniformly effective.

RESULTS

Phase 1. Five animals were implanted with the phase 1 prosthesis. Three larynges were obtained 3 months from implantation, and 2 at 6 months. With the phase 1 device, which consisted of a simple coil, 3 animals developed complications related to the implant device. At 3 months, endoscopic examination prior to laryngectomy demonstrated evidence of an endolaryngeal sinus tract in 2 animals. In 1 animal, the device was extruded externally, secondary to infection. Histologic examination confirmed endolaryngeal fistula formation in 2 animals. There was no evidence of endolaryngeal fistula in the animal with device extrusion.

In the 2 animals surviving 6 months after implantation, endolaryngeal examination demonstrated lateralization of the true vocal fold. There was no evidence of endolaryngeal fistula, and histologic examination demonstrated minimal inflammation adjacent to the implant site, with surrounding fibrosis along the coil tract (histopathologic interpretation by W. H. Westra).

Phase 2. Based on the findings in phase 1, the engaging device was designed as a double-helix screw. This device prevents the deflection of the coil as the tissue is engaged, thus providing a precise course between the thyroid lamina and the region of the vocalis process. It also provides for a broader implant-to-tissue interface.

In phase 2, 4 animals were implanted with the device. All were euthanized after 3 months. At that time, each animal was sedated with ketamine and xylazine and anesthetized with halothane-oxygen. Direct laryngoscopy was performed with a rigid laryngoscope and a 4.0-mm 0° endoscope with a video system. The endotracheal tube was removed over an Eshman stylet, and the larynx was visualized during spontaneous respiration. In all 4 animals, the right true vocal fold (the ipsilateral side) was immobile and lateralized. There was no evidence of endolaryngeal trauma, granulation tissue, or fistula. Palpation of the vocalis process revealed that the true vocal fold was stable in the lateralized position. Each animal was then euthanized with a sodium pentobarbital overdose, and the larynges were excised for gross as well as histopathologic examination. There was no evidence of infection or inflammation around the implant site (histopathologic interpretation by W. H. Westra). The larynx was split posteriorly for visualization, and there was no evidence of induced inflammation (Fig 2).

DISCUSSION

At this time, there is no procedure available that effectively and reliably allows vocal fold lateralization for the treatment of individuals afflicted with bilateral abductor paralysis. Destructive procedures such as cordectomy, arytenoidectomy, and cordotomy offer adequate results in many cases, leaving the surgeon subjectively confident of the end results. An increase in the airway at the true vocal fold level may be achieved at the termination of the surgical procedure, but establishment of a predictably enlarged airway over time remains a more difficult undertaking. Preservation of phonatory capability and avoidance of substantial aspiration remain significant unresolved issues.

The device described herein potentially provides additional benefits in human application: 1) the procedure can be performed under local anesthesia with visualization through the fiberoptic laryngoscope; 2) the placement of the prosthesis and degree of vocal fold lateralization can be continuously monitored; and 3) the device is tunable with respect to voice and airway enhancement. These 3 critical advantages speak favorably for the concept. A human study has been initiated to evaluate the effectiveness of this prosthesis in the clinical setting. This investigation will assess both the feasibility and the long-term capability of the device. All patients under investigation will have received a prior tracheotomy as treatment for their bilateral abductor impairment. Device success will be defined as decannulation with the maintenance of a satisfactory airway and reasonable voice. The suitable patient population will be those afflicted with bilateral abductor paralysis caused by neurologic accidents or disorders, surgical or traumatic injuries leading to neurologic deficits, or cricoarytenoid ankylosis.

CONCLUSIONS

1. There is not, as yet, an effective, minimally invasive procedure to incrementally lateralize a vocal fold in patients with bilateral abductor paralysis.

2. A double-helix, double-cam stainless steel–polyethylene device appears to be effective without causing substantial irreversible changes.

3. A currently ongoing human effectiveness study will establish the suitability of this concept and prosthesis in the clinical setting.

REFERENCES

1. Remsen K, Lawson W, Patel N, Biller HF. Laser lateralization for bilateral vocal cord abductor paralysis. Otolaryngol Head Neck Surg 1985;93:645-9.

2. Eckel HE, Thumfart M, Wasserman K, Vössing M, Thumfart WF. Cordectomy versus arytenoidectomy in the management of bilateral vocal cord paralysis. Ann Otol Rhinol Laryngol 1994;103:852-7.

3. Rinne J. Late results of laterofixation in the treatment of bilateral abductor paralysis of the vocal cords: a clinical study with long-term follow-up. Clin Otolaryngol 1991;16:436-41.

4. Gupta AK, Mann SB, Nagarkar N. Surgical management of bilateral immobile vocal folds and long-term follow-up. J Laryngol Otol 1997;111:474-7.

5. Merite-Drancy A, Laccourreye O, Brasnu D, Laccourreye H. Partial posterior cordectomy with laser CO2 in bilateral recurrent paralysis. Ann Otolaryngol Chir Cervicofac 1992;109:235-9.

6. Hawthorne MR, Nunez DA. Bilateral vocal cord palsy: the alternative to tracheostomy. J Otolaryngol 1992;21:364-5.

7. Kirchner FR. Endoscopic lateralization of the vocal cord in abductor paralysis of the larynx. Laryngoscope 1979;89:1779-83.

8. Kleinsasser O, Nolte E. Report of the indication, technique and functional results of endolaryngeal arytenoidectomy and submucous partial cordectomy in bilateral paralysis of vocal cord. Laryngol Rhinol Otol (Stuttg) 1981;60:397-401.

9. Wani MK, Yarber R, Hengesteg A, Rosen C, Woodson GE. Endoscopic laser medial arytenoidectomy versus total arytenoidectomy in the management of bilateral vocal fold paralysis. Ann Otol Rhinol Laryngol 1996;105:857-62.

10. Ejnell H, Mansson I, Hallen O, Bake B, Stenborg R, Lindstrom J. A simple operation for bilateral vocal cord paralysis. Laryngoscope 1984;94:954-8.

11. Kashima H. Bilateral vocal fold motion impairment: pathophysiology and management by transverse cordotomy. Ann Otol Rhinol Laryngol 1991;100:717-21.

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SUBGLOTTIC STENOSIS: CORRELATION BETWEEN COMPUTED TOMOGRAPHY AND BRONCHOSCOPY

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The evaluation of subglottic stenosis has been limited by the lack of standardized methods for determining the cross-sectional area and length of the stenotic segment. A rabbit model was used to prospectively evaluate the correlation between computed tomography (CT) and bronchoscopy in the evaluation of this disease. Subglottic stenosis was produced in 39 New Zealand White rabbits by a transoral endoscopic technique. The animals were evaluated 3 weeks later with spiral CT, rigid bronchoscopy, and open laryngotracheal exploration. Spiral CT was performed with the location, degree, and length of subglottic stenosis being determined by a blinded observer. Each animal then underwent rigid bronchoscopy and open laryngotracheal exploration for determination of the same measurements. Data were analyzed to determine the correlation between the radiographic and surgical techniques in evaluating the airway stenosis. With regard to the degree of stenosis, 94% of the rabbits were determined to have CT and bronchoscopic measurements that were within 15% (Pearson correlation .94, $p < .05$). With regard to the length of stenosis, 94% of animals had a measurement on CT that was within 2 mm of that observed upon open exploration (Pearson correlation .81, $p < .05$). The CT evaluation of subglottic stenosis correlated well with the currently used method of visual inspection at bronchoscopy in evaluating tracheal stenosis in this animal model. These data suggest that CT could serve as a useful adjunct in the evaluation of tracheal stenosis, especially when serial examinations are required.

KEY WORDS — bronchoscopy, computed tomography, evaluation, subglottic stenosis.

INTRODUCTION

The incidence of acquired subglottic stenosis in the pediatric population has risen significantly over the past 3 decades. Recent literature suggests an incidence of 2.5% in neonates subjected to long-term intubation.[1] Furthermore, 90% of cases of subglottic stenosis in children are secondary to endotracheal intubation.[2]

Several systems have been proposed for measuring stenotic lesions, including radiographic studies, endoscopy, rhinomanometry,[3] body plethysmography,[4] and flow volume loops.[5] Computed tomography (CT) has proven to be of diagnostic importance in children with airway obstruction[6]; however, many question the use of CT in the evaluation of subglottic stenosis and feel that its role is limited.[2,7-9]

Definitive endoscopic evaluation involves rigid bronchoscopy. The laryngotracheal stenosis grading system most commonly used is that proposed by Cotton et al,[10] in which the percentage of perceived obstruction and the anatomic location of the lesions are determined by endoscopic assessment. Statistical analysis has demonstrated that this system successfully predicts the prognosis for decannulation; however, some feel this method is limited by variations in measurements depending on the position of the bronchoscope during photodocumentation.[8,9,11] Various adaptations to the endoscopic evaluation of subglottic stenosis have included the use of endoscopic rulers,[12] balloon-tipped angioplasty catheters,[13] grading systems based on endotracheal tube sizes,[9] and photometrics.[8]

The purpose of this study was to evaluate the role of CT and bronchoscopy in the examination of acquired subglottic stenosis. To achieve this goal, an animal model was developed to permit the evalua-

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Presented at the meeting of the American Broncho-Esophagological Association, Palm Beach, Florida, May 11-12, 1998. Winner of the Seymour Cohen Award for Pediatric Laryngology and Broncho-Esophagology Research.

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TABLE 1. BRONCHOSCOPY VERSUS COMPUTED TOMOGRAPHY

Correlation Between Modalities Regarding Percentage of Stenosis	*Rabbits (N = 39)*	
	No.	*%*
Within 5%	12	31
Within 10%	34	87
Within 15%	37	95
Within 20%	39	100

tion of subglottic stenosis comparing spiral CT, rigid bronchoscopy, and open laryngotracheal exploration. Subsequently, data were analyzed to determine the correlation between the radiographic and surgical techniques in evaluating tracheal stenosis.

METHODS

Subglottic stenosis was produced in 39 New Zealand White rabbits (13 to 15 weeks of age) by a transoral endoscopic technique. Each animal was anesthetized with an intramuscular injection of ketamine hydrochloride (30 mg/kg) and xylazine hydrochloride (6 mg/kg). All animals were allowed to breathe spontaneously without the use of an artificial airway. The rabbit was placed supine with the ears tucked and the neck extended. Direct laryngoscopy was performed and vocal fold spasm was prevented with topical lidocaine. A 0° Hopkins rod was passed into the airway and the supraglottic, glottic, and subglottic regions were examined for any preexisting abnormalities. Once these had been excluded, the telescope was removed. Depending on the native airway size, a 5-, 6-, or 7-mm bur was passed into the subglottic region and turned 5 to 6 times in an effort to traumatize the subglottic lumen at the cricoid level.

The animals were monitored postoperatively for any signs or symptoms of acute respiratory distress, mediastinitis, sepsis, or air hunger. Appropriate lodging and daily care were provided by certified animal caretakers. The animals were evaluated 3 weeks later with spiral CT, rigid bronchoscopy, and open laryngotracheal exploration. Spiral CT was performed on contiguous 1-mm-thick sections with 1-mm pitch extending from the mandible to the clavicles. The protocol required 120 kV (peak), 200 mA, and 0.75 second per slice. The approximate dose of radiation was 9.15 milligrays to the trachea and 17.4 milligrays to the skin. The degree of subglottic stenosis was determined by measuring the cross-sectional area of the stenotic tracheal lumen on axial images at the point of maximal stenosis. The cross-sectional area of the normal tracheal lumen was determined by measuring a tracheal section 3 mm below the inferior extent of the stenosis. The percentage of stenosis was calculated by comparing these two values. The length of stenosis was determined by counting the number of contiguous axial images demonstrating stenosis.

Each animal then underwent rigid bronchoscopy and open laryngotracheal exploration. A blinded observer determined the extent of subglottic stenosis by specifying the anatomic location, and estimating the percent of perceived tracheal stenosis during endoscopy (with the estimation being a multiple of 10). The length of the stenotic tracheal segment was determined with open laryngotracheal exploration. Intraoperative and radiographic data were analyzed to determine the correlation between the radiographic and surgical techniques in evaluating the airway stenosis. Statistical analysis was performed with the Pearson product moment correlation test; $p < .05$ was considered statistically significant.

RESULTS

Intraoperative and radiographic measurements of the *degree* of stenosis demonstrated good correlation (Pearson correlation .94, $p < .05$). Thirty-one percent (12/39) of the rabbits had CT and bronchoscopy measurements that were within 5 percentage points. Eighty-seven percent (34/39) of measurements were determined to be within 10 percentage points, and 95% (37/39) were determined to be within 15 percentage points. All rabbits had radiographic and intraoperative findings that were within 20 percentage points (Table 1). Seven of 10 animals with stenoses between 30% and 50%, 19 of 20 animals with stenoses between 51% and 70%, and 8 of 9 animals with stenoses between 71% and 100% had degree measurements that were within 10 percentage points. Figures 1 and 2 demonstrate correlation between endoscopy and radiographic measurements.

The *length* of stenoses ranged from 2 to 5 mm, and the findings on CT correlated well with the measurements made during open laryngotracheal exploration (Pearson correlation .81, $p < .05$). Seventy-seven percent (30/39) of rabbits had measurements that were within 1 mm, while measurements were within 2 mm in 95% of rabbits (37/39). All animals had measurements that were within 3 mm (Table 2). Finally, 6 of 10 animals with stenoses between 30% and 50%, 17 of 20 animals with stenoses between 51% and 70%, and 7 of 9 animals with stenoses between 71% and 100% had length measurements that were within 1 mm.

DISCUSSION

Numerous methods have been developed to assess the degree of subglottic stenosis. Radiographic studies have included soft tissue x-rays of the neck, including lateral and high-kilovoltage anteroposterior projections; fluoroscopy; and xeroradiography.[7] Other proposed methods have included an adapted

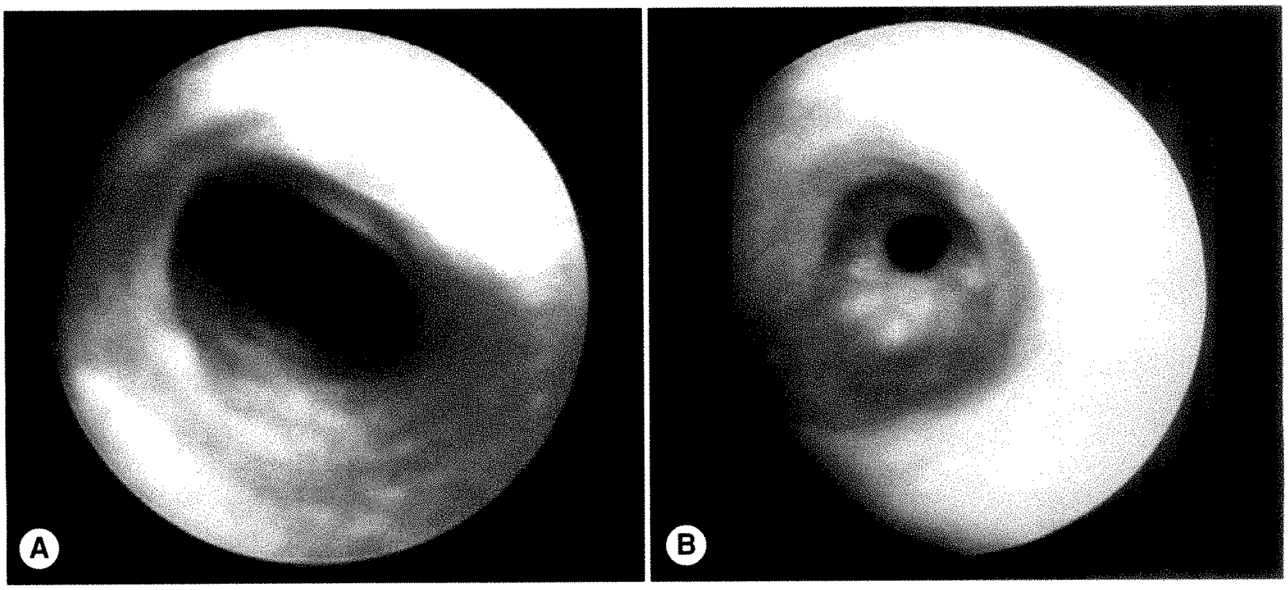

Fig 1. Views of rabbit tracheas through 0° Hopkins rod endoscope. A) Normal rabbit. B) Rabbit with subglottic stenosis.

rhinomanometer,[3] body plethysmography,[4] and flow volume loops.[5] All provide useful information, but none has gained widespread acceptance as the most reliable modality for objectively measuring the degree of subglottic stenosis.

Both flexible and rigid endoscopy have been recommended for the endoscopic evaluation of subglottic stenosis. Flexible fiberoptic endoscopy assesses the dynamics of vocal cord function and provides an initial impression of subglottic disease. However, for a definitive assessment of tracheal stenosis, a child must be anesthetized and the airway examined with a rigid optical telescope.[7] The caliber of stenosis has been assessed by passing various sizes of bronchoscopes through the stenotic lumen and determining the ease with which the bronchoscope passes.[7] However, judging the extent of a stenosis by the external diameter of a bronchoscope is open to considerable interobserver variation.[8,9,11] In addition, some cases of severe stenosis will not allow passage of the smallest bronchoscope. Studies indicate that the decision to decannulate based on this method is correct only 64% to 89% of the time.[8]

The degree of subglottic stenosis can also be determined by estimating the percentage of subglottic lumen compromised by scar tissue through visual inspection; however, this method may also demonstrate variation between observers.[8,9,11] Jones et al[8] have attempted to improve this method with the application of photometrics. By standardizing the distance from the objective of the endoscope to the stenotic area and graduating the optical field at that distance, endoscopic measurements were made of the cross-sectional area of a stenotic rubber tube. These authors found that when measuring the minimum cross-sectional area, 3 of 5 observers were significantly

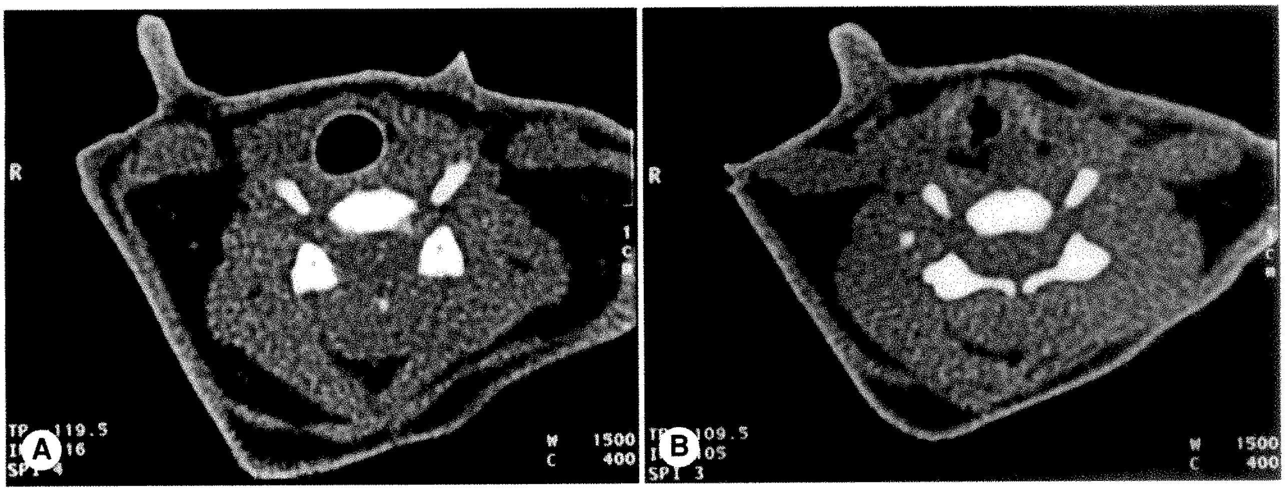

Fig 2. Axial computed tomograms of rabbit tracheas. A) Normal rabbit. B) Rabbit with subglottic stenosis.

TABLE 2. COMPUTED TOMOGRAPHY VERSUS BRONCHOSCOPY AND OPEN EXPLORATION

Correlation Between Modalities Regarding Length of Stenosis	*Rabbits (N = 39)* No.	%
Within 1 mm	30	77
Within 2 mm	37	95
Within 3 mm	39	100

better at using the photometric method than the bronchoscope method.[8] Despite these promising results, the same results have not been demonstrated in animal or clinical studies. Other methods associated with endoscopic evaluation of laryngotracheal stenosis have included endoscopic rulers,[12] balloon-tipped angioplasty catheters,[13] and grading systems based on endotracheal tube sizes.[9]

In 1988, the American Society of Pediatric Otolaryngologists adopted a system known as FLECS, for function, lumen, extent, consistency, and site of laryngotracheal stenosis. Determining the degree of stenosis as well as the maturity and consistency of the lesion was emphasized. Standard data collection forms were distributed to otolaryngologists to guide their use of the FLECS system; however, most felt that the system was too cumbersome and did not use it routinely. As a result, no information has been published that was gained from the use of this system.

The laryngotracheal stenosis grading system most commonly used is that proposed by Cotton[14] in 1984, with its subsequent modifications by Cotton et al[10] in 1989. The percentage of obstruction and anatomic location of the lesions are determined by endoscopic assessment, and grades I to IV are assigned. Although this system is still dependent on subjective interpretation, statistical analysis has demonstrated that the system successfully predicts the prognosis for decannulation.

Computed tomography assessment of tracheal anatomy and diameter has proven to be of diagnostic importance for children with airway obstruction, especially when evaluating tracheal compression secondary to an extraluminal mass.[6] In addition, Lee et al[15] demonstrated good correlation between helical CT and bronchoscopy in the evaluation of distal tracheobronchial disease. However, many have felt that the use of CT in the diagnostic evaluation of subglottic stenosis is limited.[2,7-9] Faw et al[11] evaluated the role of CT by examining 3 neonatal larynges that had been removed at necropsy. They determined that CT provided good resolution of soft tissue, cartilage, and the airway lumen. Subsequently, 2 neonates with acquired subglottic stenosis were studied and radiographic and endoscopic examinations demonstrated good correlation.[11]

Hernandez and Tucker[16] evaluated the role of chest radiographs, high-kilovoltage films, CT, and bronchoscopy in 5 patients with congenital subglottic stenosis. They noted that CT was superior to bronchoscopy in the evaluation of the distal extent of the stenosis in those patients who had such tight stenosis that the bronchoscope could not be passed through the stenotic lumen. This study also determined that CT was useful in ruling out external compression of the trachea by a mass or vascular anomaly.[16]

This study was designed to evaluate the correlation between CT and bronchoscopy in the examination of acquired subglottic stenosis. Endoscopic evaluation is recognized as the most commonly used method for assessing the extent of subglottic stenosis, especially since the introduction of Cotton's classification system. First of all, endoscopy is helpful in looking for any concomitant laryngotracheal disease, including dynamic airway lesions and posterior glottic stenosis. Second, endoscopic evaluation is essential in assessment of the consistency of scar tissue at the stenotic site, differentiating soft, pliable tissue from firmly organized scar tissue. The history of the injury and the findings at endoscopy are essential in making this distinction. Finally, endoscopic evaluation is necessary to familiarize the surgeon with the patient's airway anatomy prior to any immediate or future open surgical procedures. Nonetheless, some have questioned the use of these methods as the sole instrument in the examination of the airway.[8,9,11] The degree of perceived stenosis may vary depending on the distance between the bronchoscope and the lesion. This fact is extremely important when photodocumentation is being used to facilitate communication between different physicians or for comparing changes in a lesion over various time periods. Thus, some surgical centers have adopted techniques such as photometrics, endoscopic rulers, or endotracheal tube grading systems in an attempt to standardize the measurements made at endoscopy.

In short, endoscopic evaluation of subglottic stenosis is essential in the initial evaluation of a patient with subglottic stenosis in order to assess the consistency of the scar tissue, to evaluate the airway for concomitant disease, and to measure the degree of subglottic stenosis. In addition, endoscopy often needs to be repeated in the perioperative period in patients undergoing laryngotracheal reconstruction. Nonetheless, CT may be a useful adjunct to endoscopy in documenting changes between serial examinations, in evaluating the length of severely stenotic lesions, and in assessing the airway for decannulation. We recognize that CT examination is most plausible in patients having a tracheotomy or another form of secured airway.

CONCLUSION

The importance of this work is that we have demonstrated, through a controlled, prospective animal study, that CT evaluation of subglottic stenosis correlates with the stenosis found at endoscopy and open surgical exploration. Although the clinical application may be limited, spiral CT appears to be a reproducible, objective method that could serve as a useful adjunct to bronchoscopy in the evaluation of subglottic stenosis.

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REFERENCES

1. Nicklaus PJ, Crysdale WS, Conley S. Acquired neonatal subglottic stenosis. Arch Otolaryngol Head Neck Surg 1990; 116:902-4.
2. Healy G. Subglottic stenosis. Otolaryngol Clin North Am 1989;22:599-606.
3. Kearns DB, Albert DM, Choa DI, Sickstead M, Bailey CM, Evans JNG. Functional assessment of the paediatric laryngeal airway. Clin Otolaryngol 1988;15:53-8.
4. Forte V, Cole P, Crysdale WA. Objective assessment of upper airway resistance in the tracheostomised patient. J Otolaryngol 1986;15:359-61.
5. Mallory GB Jr, Reilly JS, Motoyama EK, Mutich R, Kenna MA, Stool SE. Tidal flow measurement in the decision to decannulate the pediatric patient. Ann Otol Rhinol Laryngol 1985;94:454-7.
6. Effmann EL, Fram EK, Vock P, Kirks DR. Tracheal cross-sectional area in children: CT determination. Radiology 1983; 149:137-40.
7. Cotton RT. Management and prevention of subglottic stenosis in infants and children. In: Bluestone CD, Stool SE, eds. Pediatric otolaryngology. 2nd ed. Philadelphia, Pa: WB Saunders, 1990:1190-204.
8. Jones NS, Irving RM, Ford BG, Balakrishnan A, Albert DM. Photometrics: a new method of measuring the cross-sectional area of the subglottis. Clin Otolaryngol 1992;17:141-5.
9. Myer CM III, O'Connor DM, Cotton RT. Proposed grading system for subglottic stenosis based on endotracheal tube sizes. Ann Otol Rhinol Laryngol 1994;103:319-23.
10. Cotton RT, Gray SD, Miller RP. Update of the Cincinnati experience in pediatric laryngotracheal reconstruction. Laryngoscope 1989;99:1111-6.
11. Faw K, Muntz H, Siegel M, Spector G. Computed tomography in the evaluation of acquired stenosis in the neonate. Laryngoscope 1982;92:100-5.
12. Toohill RJ, Campbell BH. An endoscopic ruler. Otolaryngol Head Neck Surg 1986;94:134-5.
13. Hebra A, Powell DD, Smith CD, Othersen HB Jr. Balloon tracheoplasty in children: results of a 15-year experience. J Pediatr Surg 1991;26:957-61.
14. Cotton RT. Pediatric laryngotracheal stenosis. J Pediatr Surg 1984;19:699-704.
15. Lee KS, Yoon JH, Kim TK, Kim JS, Chung ME, Kwon OJ. Evaluation of tracheobronchial disease with helical CT with multiplanar and three-dimensional reconstruction: correlation with bronchoscopy. Radiographics 1997;17:555-67.
16. Hernandez RJ, Tucker GF. Congenital tracheal stenosis: role of CT and high kV films. Pediatr Radiol 1987;17:192-6.

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PALLIATIVE TREATMENT FOR TRACHEAL STENOSES USING CARBON DIOXIDE LASER AND THE GIANTURCO STENT

LONG-TERM RESULTS

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Between September 1992 and March 1998, the self-expandable Gianturco prosthesis was inserted in 23 patients suffering from tracheal stenosis. After radial incision and dilation of the stenosis as described by Shapshay, the positioning of the stent was performed during an endoscopic procedure under optical control. The prosthesis used was a double-ring stent 50 mm long and 20 mm in diameter. The follow-up period ranged between 0.5 and 67 months with an average of 31 ± 18 months. Pulmonary function tests showed an average improvement of the peak expiratory flow (50%) from preoperative results of 1.06 ± 0.60 L/s to short-term postoperative results of 2.08 ± 0.78 L/s and long-term postoperative results of 2.11 ± 0.78 L/s. The mean peak inspiratory flow (50%) improved from 1.43 ± 0.85 L/s to 2.40 ± 1.29 L/s at short term and to 2.56 ± 1.20 L/s at long term. Eight patients out of the 23 had to undergo a second endoscopic procedure: 3 patients for granuloma vaporization; 1 patient to change a malpositioned stent; 2 patients to add a second stent because of insufficient tracheal enlargement; and 2 patients to resect mucosal membranes between the 2 stent rings and to place a second stent. Optical control of the accurate positioning and use of this model of Gianturco prosthesis helped to avoid the severe complications described in the literature (migration, extrusion, fracture, wall erosion, and hemorrhage). The follow-up must particularly target the prevention of granulomas. The self-expandable Gianturco prosthesis can be advocated for long-term palliative treatment of tracheal stenoses that are inoperable by an external surgical approach.

KEY WORDS — laser, palliative treatment, stent, tracheal stenosis.

INTRODUCTION

The use of stents for endoscopic treatment of tracheal stenoses has developed during the past 10 years.[1] The first stents used were silicone models for the palliative treatment of tracheobronchial tumors.[2] Pertaining to that particular indication, many authors affirm their choice of such stent models.[3] Later, metallic stents were introduced: the nonexpandable wire mesh Strecker stent,[4] and the titanium alloy nitinol stent "with thermal shape memory," so termed because it returns to its shape once cooled.[5] Some stents are expandable and made of metallic wire, such as the Gianturco stent[6] and the Wall stent.[7] Other stents are balloon-expanded, such as the Palmaz stent[8] (also made of metallic wire).

There are several advantages to metallic wire stents[9]: they can be positioned during an endoscopic procedure under general anesthesia or by fluoroscopic guidance under local anesthesia; they do not impede the drainage of secretions, because the walls of the stent are not solid; and the stents are dynamic and imitate tracheal movements. The stents also have disadvantages: without regular care, granuloma formation frequently occurs; the stents are difficult to withdraw; and the stents cannot be used in the event of tumors. Complications have been reported: migration,[10] rupture,[11] and wall[12] and vessel[13,14] perforation.

Freitag et al[15] proposed the use of a silicone stent in the wall of which are incorporated steel horseshoe-shaped struts making the stent more physiological. Tsang et al[16] proposed combining the Wall stent and the silicone stent. Since the publication of their article, the silicone-covered Wall stent has been developed, and it is mainly indicated in cases of tumoral stenosis.

We report our long-term experience in combining carbon dioxide (CO2) laser dilation and the Gianturco stent for palliative treatment of tracheal stenoses.

MATERIALS AND METHODS

From September 1, 1992, to March 30, 1998, we inserted the Gianturco prosthesis in 23 patients suf-

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TABLE 1. CHARACTERISTICS OF 23 PATIENTS WITH TRACHEAL STENOSIS TREATED WITH GIANTURCO STENT

Patient No.	*Sex*	*Age (y)*	*Causes of Stenosis*	*Contraindications for External Surgery*
1	M	54	Tracheotomy	Hydrocephalus
2	M	74	Tracheotomy	Tracheomalacia, obesity, sleep apnea syndrome
3	F	68	Tracheotomy	Tracheomalacia
4	F	59	Radiotherapy	Thyroid gland carcinoma treated with radiotherapy
5	M	68	Prolonged intubation	Aortic aneurysm, tracheomalacia, obesity
6	M	63	Tracheotomy	Chronic obstructive lung disease
7	F	70	Prolonged intubation	Pulmonary tuberculosis
8	F	68	Tracheotomy	Major cardiac risk
9	M	65	Tracheotomy and prolonged intubation	Major cardiac risk
10	M	65	Prolonged intubation	Major cardiac risk
11	M	57	Radiotherapy and tracheotomy	Laryngeal carcinoma treated with radiotherapy
12	F	63	Prolonged intubation	Chronic obstructive lung disease
13	F	72	Prolonged intubation	Major cardiac risk, chronic obstructive lung disease, nervous breakdown
14	F	59	Prolonged intubation	Major cardiac risk, chronic obstructive lung disease, diabetes
15	M	64	Tracheotomy and prolonged intubation	Major cardiac risk, chronic obstructive lung disease
16	M	49	Prolonged intubation	Chronic obstructive lung disease
17	M	49	Prolonged intubation	Major cardiac risk, obesity
18	M	69	Extrinsic compression	Major cardiac risk
19	F	67	Multiple tracheotomies	Diabetes, obesity, renal insufficiency
20	F	83	Extrinsic compression	Age, breast cancer, thyroid gland carcinoma, refusal of invasive treatment
21	F	67	Prolonged intubation	Tracheomalacia, chronic respiratory insufficiency, right pneumonectomy for tuberculosis
22	F	64	Prolonged intubation	Cardiopathy
23	M	60	Prolonged intubation	Refusal for external surgery

fering from tracheal stenosis (11 women and 12 men; Table 1). The patients' ages ranged between 49 and 83, with an average of 64 ± 5.7 years (median 65). The causes of the stenoses were a difficult tracheotomy and/or prolonged intubation, radiotherapy, and external compression. All patients would have been at high risk of complications if they had undergone an external surgical approach, be it resection-anastomosis or tracheoplasty. The first 11 patients were already described in our first report on this topic in July 1994.[17] We therefore continued using the Gianturco stent, which, on the one hand, is self-expandable and, on the other hand, is easily placed via an endoscopic approach. Twelve patients were added to the list in the following 4 years. Outside of this group we also used the Unistent 20/48 Wall stent prosthesis for a 60-year-old woman patient suffering from tracheomalacia and sleep apnea syndrome. In absolute values, preoperative pulmonary function tests show (Table 2) a mean peak expiratory flow (PEF 50%) of 1.06 ± 0.60 L/s (median 0.85) with a minimum of 0.22 and a maximum of 2.27; and a mean peak inspiratory flow (PIF 50%) of 1.43 ± 0.85 L/s (median 1.08) with a minimum of 0.54 and a maximum of 3.22. The average preoperative PIF 50%/PEF 50% ratio is 1.99 ± 3.10 (median 1.31), with a minimum of 0.56 and a maximum of 13.91.

The Gianturco prosthesis used is always the double-ring prosthesis that is 50 mm long and has a 20-mm diameter after expansion. Since 1996, we have had at our disposal a new model with the rings attached to each other at the level of each opposite fold. Previously, a single bridge attached both rings to each other.

Preoperative assessment continues to be based on fiberoptic endoscopy, pulmonary function tests, and computed tomography (CT).

The surgical technique remains the same: the operation begins with an examination of the trachea using a rigid fiberoptic endoscope (Fig 1A). Then a bronchoscope that is adapted to CO_2 laser surgery (Broncholaser, Sharplan Laser Industries) is positioned facing the stenosis. Ventilation is ensured by a jet ventilation catheter connected to a canal of the bronchoscope (AMS-100, Acutronic Medical Systems AG, Switzerland). In the case of a fibrous stenosis, the operation begins with a radial incision as described by Shapshay et al,[18] in pulsed mode at 10-W power. The bronchoscope is then passed through

TABLE 2. RESPIRATORY FUNCTIONAL RESULTS

	Peak Expiratory Flow (50%) (L/s)			*Peak Inspiratory Flow (50%) (L/s)*		
Patient No.	*Preoperative*	*Postoperative*	*Last Control*	*Preoperative*	*Postoperative*	*Last Control*
1	0.69	2.36	2.81	0.75	1.09	3.09
2	0.46	1.08	1.50	1.08	1.52	1.41
3	NP	2.57	3.04	NP	3.59	3.28
4	0.78	1.96	2.53	0.78	2.20	1.87
5	0.85	1.12	0.84	1.31	2.43	1.69
6	0.22	0.69	NP	3.06	1.92	NP
7	NP	1.78	NP	NP	1.42	NP
8	1.60	2.89	2.01	2.10	2.82	2.60
9	1.60	2.59	2.06	3.22	7.13	5.91
10	1.09	3.70	3.07	1.50	3.30	2.62
11	1.30	1.65	TL	0.92	2.20	TL
12	0.71	2.55	2.61	0.95	1.96	1.64
13	0.54	2.77	NP	0.54	2.18	NP
14	0.62	0.96	0.70	0.89	1.72	2.19
15	NP	1.80	NP	NP	3.41	NP
16	NP	1.57	1.52	NP	2.67	2.42
17	2.21	3.04	2.62	2.44	1.82	TL
18	1.54	2.84	TL	2.16	2.37	TL
19	0.67	NP	NP	0.71	NP	NP
20	0.88	1.82	NP	0.61	1.47	NP
21*	NP	NP	NP	NP	NP	NP
22	2.27	2.38	2.16	1.27	2.19	1.96
23	NP	1.70	NP	NP	1.07	NP

NP — not performed, TL — total laryngectomy.
*Maintained tracheostomy.

the stenosis in order to dilate it (Fig 1B). Dilation is assessed as sufficient when a 9-mm–outer diameter bronchoscope can be passed without difficulty. Dilation can be initiated with a smaller-diameter bronchoscope. Afterward, the prosthesis, which is placed within the bronchoscope, is pushed with a 460-mm-long by 3.5-mm-diameter telescope (Wolf, Knittlingen, Germany). This telescope allows visual control for the correct positioning of the stent (Figs 1C and 2).

It is important to ensure that neither dried blood nor accumulated secretions, which form during dilation, soil the lumen of the bronchoscope. Such deposits can considerably interfere with the sliding of the prosthesis through the bronchoscope. The release of the prosthesis begins at the inferior end of the stenosis. Then, while the bronchoscope is slowly withdrawn, the endoscope maintains the pressure on the prosthesis. Adjustments can be made with the help of microforceps. If, however, the positioning is inadequate, then the traction movements applied by the microforceps will buckle the stent by unfolding the metallic wire. The stent must then be entirely removed by pulling it through the bronchoscope, and one must restart the maneuver with a new stent.

Before surgery, intravenous antibiotic and steroid therapy is initiated. Afterward, the treatment is continued orally and by aerosols for 5 to 6 days. Depending on postoperative fiberoptic controls, the treatment, if necessary, can be prolonged.

Immediate postoperative controls include pulmonary function tests as well as neck and chest X-rays (Fig 3) to verify the proper position of the prosthesis. The prosthesis must not be painful to the patient. The patient must be able to breathe and swallow without difficulty. If this is not the case, then a malposition must be suspected. If, before surgery, the patient is tracheotomized, it is preferable not to close the aperture at the same time as the placement of the stent. However, 1 or 2 days is sufficient to make sure that the placement is absolutely efficient; and we no longer advocate a 15-day delay.

RESULTS

Immediate follow-ups were straightforward for all patients (Table 3), an exception being patient 6, who, as previously reported, underwent, 15 days after the first endoscopy, a change of stent because of a malposition.

Patients 1, 17, and 23 underwent surgery for granuloma vaporization. In the case of patients 5 and 14,

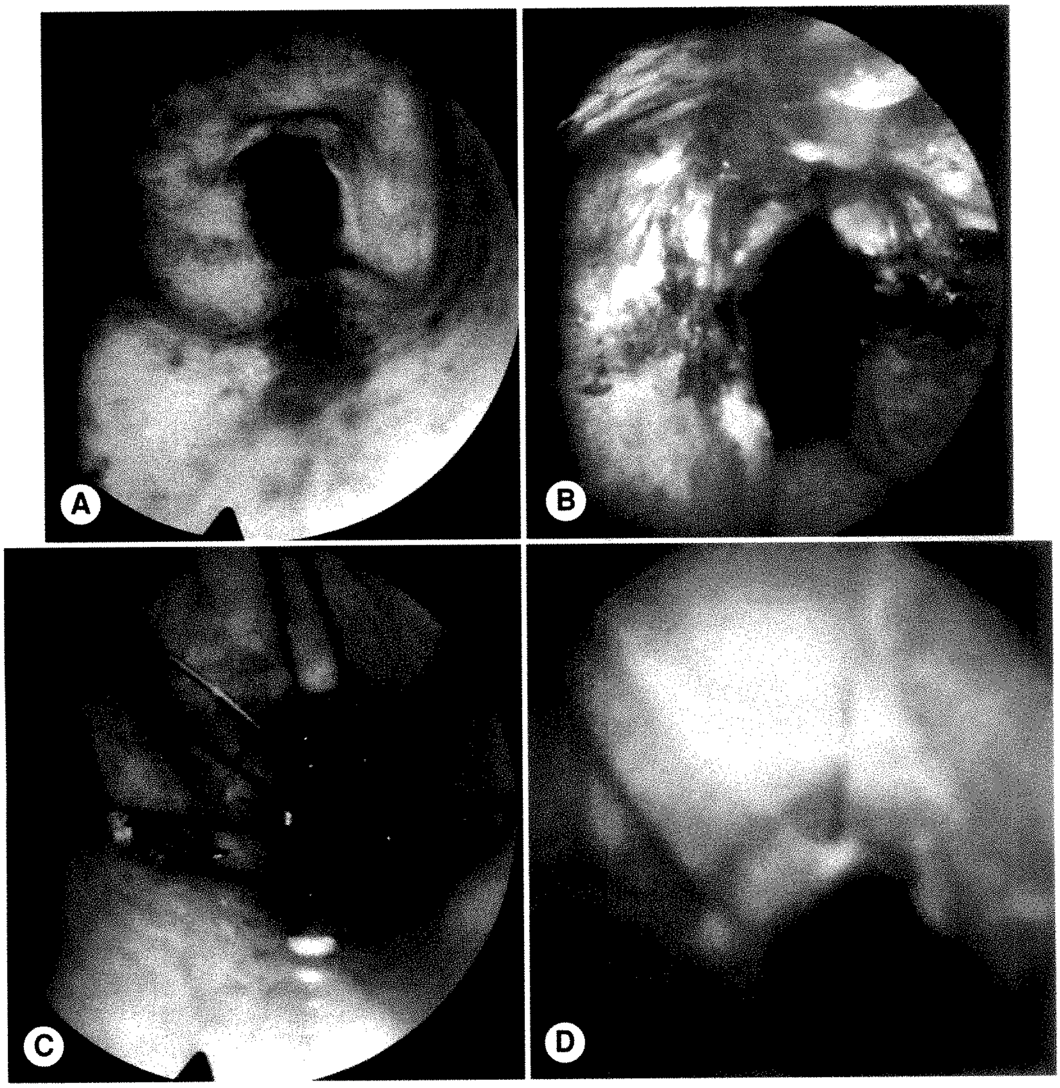

Fig 1. Endoscopic views. A) Tracheal stenosis. B) Quadrant incision according to Shapshay and bronchoscope dilation. C) Gianturco stent in place. D) Fiberscopy after 2 weeks. Stent is partially covered by inflammatory tissue.

a second double-ring prosthesis was positioned because of insufficient enlargement. In such cases, the second prosthesis is placed within the lumen of the first prosthesis in order to reinforce expansion.

Patients 7 and 17 had to undergo laser resection of a mucosal membrane that had developed between the 2 rings of the first prosthesis. In patient 7, a single-ring prosthesis was placed at the site of the membrane; patient 17 required the placement of a double-ring prosthesis.

Patients 11 and 18 underwent total laryngectomy after placement of the stent. The cause of the tracheal

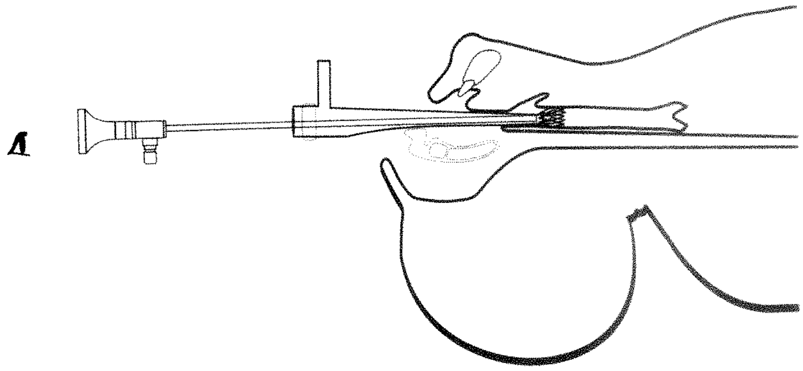

Fig 2. Drawing of stent positioning under visual control.

Fig 3. Chest X-ray for stent control.

nosis of patient 11 was radiotherapy combined
th tracheotomy following laryngeal cancer. The
ncer recurred 12 months after the placement of the
nt. Patient 18 suffered from a thyroid cancer that
ıs infiltrating the larynx and compressing the tra-
ea. The stent had been temporarily placed in order
improve the acute dyspnea and to avoid a tracheot-
ıy in a neoplastic area.

On March 31, 1998, the follow-up period (Table
ranged from 0.5 to 67 months, with an average of
± 18 months (median 28). The female patient who
l the Wall stent showed a 21-month complication-
e follow-up. The follow-up was ended by death
4 patients and by total laryngectomy in 2 patients.

Pulmonary function tests (Tables 2 and 4) reveal,
ıbsolute values, an immediate postoperative mean
F (50%) of 2.08 ± 0.78 L/s (median 1.96) with a
nimum of 0.69 and a maximum of 3.70. The long-
n PEF 50% is considered to be the value at the
t control (of which the period varies from one pa-
ıt to another): 2.11 ± 0.78 L/s (median 2.16) with
ninimum of 0.70 and a maximum of 3.07. The
nediate PIF 50% is 2.40 ± 1.29 L/s (median 2.19),
h a minimum of 1.07 and a maximum of 7.13.
long-term PIF 50% is 2.56 ± 1.20 L/s (median
1), with a minimum of 1.41 and a maximum of
1.

The average postoperative PIF 50%/PEF 50% ratio is 1.28 ± 0.68 (median 0.98), with a minimum of 0.46 and a maximum of 2.78 for the immediate postoperative control. For the last control, the average PIF 50%/PEF 50% ratio is 1.43 ± 0.83 (median 1.09) with a minimum of 0.63 and a maximum of 3.13. According to the Wilcoxon signed rank test, those values are statistically superior to the preoperative values ($p < 0.01$).

DISCUSSION

From the stent insertion point of view, we remain in favor of positioning through a bronchoscope under visual control. We are convinced that one of the reasons for defective positioning is the lack of good vision during the release of the prosthesis. After dilation, it is difficult with fluoroscopy to assess the aspect of the stenosis. It is for that reason that we do not use the placement set provided by the manufacturer. We had taken note of the release bronchoscope designed for the Wall stent prosthesis.[19] But here also, the release cannot be performed under visual control, and fluoroscopy is necessary. The Wall stent prosthesis can be positioned in the same fashion as the Gianturco prosthesis by pushing it with an endoscope, but the Wall stent metallic wires are finer and the edges, under pressure, can bend and buckle in the lumen.

In the case of a fibrous stenosis, prior dilation, as described by Shapshay et al,[18] facilitates the placement of the prosthesis. Further, the benefit is immediate at the level of the respiratory tract. Without dilation, a period of 2 to 3 weeks is necessary for a good expansion.[20]

In regard to laser type, the CO_2 laser appears to us to be the best choice because of its low thermal effects versus the neodymium:yttrium-aluminum-garnet laser or even the potassium titanyl phosphate laser.[21]

A single-ring prosthesis can easily be dislodged and slide over or under the stenosis,[22] whereas the double-ring stent, 50 mm long by 20 mm in diameter, appears to us as the safest. Its length allows good support on the tracheal wall at either end of usual stenoses. Its 20-mm diameter, after expansion, adapts to the natural diameter of the trachea, which is 16 mm.[23] Indeed, the manufacturer advocates the use of a stent that is 15% to 25% larger than the size of the lumen at the application point. It can even be 25% to 50% larger in tight stenosis cases. But we fear that the use of a 30-mm-diameter stent is one of the causes of extrusion described in the literature. If dilation is insufficient (2 cases in 23), we reinforce the device by using a second prosthesis of the same diameter, which will increase the strength opposing

TABLE 3. TREATMENT, COMPLICATIONS, AND FOLLOW-UP OF 23 PATIENTS WITH TRACHEAL STENOSIS TREATED WITH GIANTURCO STENT

Patient No.	*Previous Treatment*	*Treatment*	*Complications*	*Delay (mo)*	*Treatment of Complications*
1	CO_2 laser	Laser and double stent	Granuloma, twice	2 and 4	Laser vaporization
2	Montgomery tube for 11 y	Double stent			
3	Mongomery tube for 10 y	Double stent			
4		Laser and double stent			
5		Laser and double stent	Insufficiency	0.3	Second double stent
6		Laser and double stent	Malpositioned stent	0.5	Replacement of stent
7		Laser and double stent	Mucosal membrane webbing	2	Vaporization and second single stent
8		Laser and double stent			
9		Laser and double stent			
10	CO_2 laser	Laser and double stent			
11		Laser and double stent			
12		Laser and double stent			
13		Laser and double stent			
14		Laser and double stent	Insufficiency	1.5	Second double stent
15		Laser and double stent			
16	CO_2 laser	Laser and double stent			
17	CO_2 laser	Laser and double stent	Granuloma, mucosal membrane webbing	5 (granuloma), 13 (webbing)	Laser vaporization (granuloma), second double stent (webbing)
18		Double stent			
19	Dilations, Dumon tube	Laser and double stent			
20		Double stent			
21	Tracheotomy	Laser and double stent			
22		Laser and double stent			
23	Tracheotomy	Laser and double stent	Granuloma	7	Laser vaporization

the stenosis without risking too much enlargement and an extrusion hazard.

In contrast with solid-wall prostheses, the prostheses with metallic wires do not oppose the drainage of secretions. They must, however, be reserved for benign stenoses. They have been used in tumoral obstruction cases,[10] but their use in such an indication appears to us as less than advisable, because they do not oppose tumoral proliferation within the lumen. In such cases, expandable solid-wall prostheses appear to us to be more effective. The advantage of expandable prostheses versus silicone solid-wall prostheses is their better adaptation to important movements of the cervical trachea such as coughing. The Dumon prostheses have proved their effectiveness for dilation of bronchial tumors or inferior tracheal tumors near the carina. However, they are less stable at the cervical level.[24]

Two or 3 weeks after the placement, inflammatory tissue partially covers the metallic wires (Figs 1D and 4). If necessary, it is always possible to remove the prosthesis, even if it is not easy.[8,20,25] The stent must be held by microforceps and then pulled through the bronchoscope. Traction will unfold the rings. Bleeding, which is easily controlled, occurs because of the microhooks fixing the prosthesis or because of mobilized inflammatory tissue. If necessary, one can partially vaporize the covering tissue before withdrawing the prosthesis.

The 2 main long-term problems encountered are granuloma formation (3 cases in 23; Fig 5) and mucous membrane development between the 2 rings (2 cases in 23). Possible granuloma formation makes it compulsory to ensure a careful follow-up with regular fiberoptic controls. We advocate 1 fiberoptic control every 3 months. The use of steroid aerosols is advised in the event of the formation of small granulomas. The more voluminous granulomas that are symptomatic at a respiratory level are vaporized by laser during endoscopy (3 cases in 23). The risk of developing a mucous membrane is less important, since we have at our disposal the new prosthesis with the 2 rings linked to each other in 2 places. The risk was more important when the rings were only attached

TABLE 4. RATIO OF PEAK INSPIRATORY FLOW (50%) TO PEAK EXPIRATORY FLOW (50%)

Patient No.	*Preoperative*	*Postoperative*	*Last Control*
1	1.09	0.46	1.1
2	2.35	1.41	0.9
3	NP	1.40	1.1
4	1.00	1.12	0.7
5	1.54	2.17	2.0
6	13.91	2.78	NP
7	NP	0.80	NP
8	1.31	0.98	1.3
9	2.01	2.75	2.9
10	1.38	0.89	0.9
11	0.71	1.33	NP
12	1.34	0.77	0.6
13	1.00	0.79	NP
14	1.44	1.79	3.1
15	NP	1.89	NP
16	NP	1.70	1.6
17	1.10	0.60	NP
18	1.40	0.83	NP
19	1.06	NP	NP
20	0.69	0.81	NP
21	NP	NP	NP
22	0.56	0.92	0.9
23	NP	0.63	NP

NP — not performed.

to each other by a single bridge.

Although we observed inflammatory granulomas, we did not note an increased amount of secretions or any superinfection directly caused by the prosthesis. We therefore did not swab secretions for bacteriologic culture.

Patients 4 and 11 did not suffer from radiotherapy-induced complications. It appears, therefore, that radiotherapy is not contraindicated. We wish to stress, however, that only patient 4 underwent direct tracheal irradiation, while patient 11 was mainly irradiated on the larynx.

The Gianturco prosthesis is not indicated for subglottic stenosis. The upper end of the prosthesis must not project beyond the inferior edge of the cricoid cartilage. Dilation of a subglottic stenosis would place the upper extremity of the prosthesis directly under or between the vocal folds. This situation would damage the vocal folds and could jam the cricoarytenoid joints. Once in place, the prosthesis must be painless and not hinder deglutition. If the contrary occurs, one must suspect a defective positioning, which, in turn, must be rectified.

The absolute values (Table 2) of the pulmonary function tests reveal good stability of the long-term results of inspiratory and expiratory levels. The ratio between the PIF 50% and the PEF 50% is close to the normal value of 0.8 before and after treatment. This is because both measures are equally disrupted in the case of a lower cervical tracheal or thoracic obstruction, and the measures are equally improved after treatment. The measure of that ratio is particularly useful in distinguishing an upper, extrathoracic airway obstruction from a lower, intrathoracic airway obstruction. The measure is less relevant in cases of stable lower tracheal stenosis.[26]

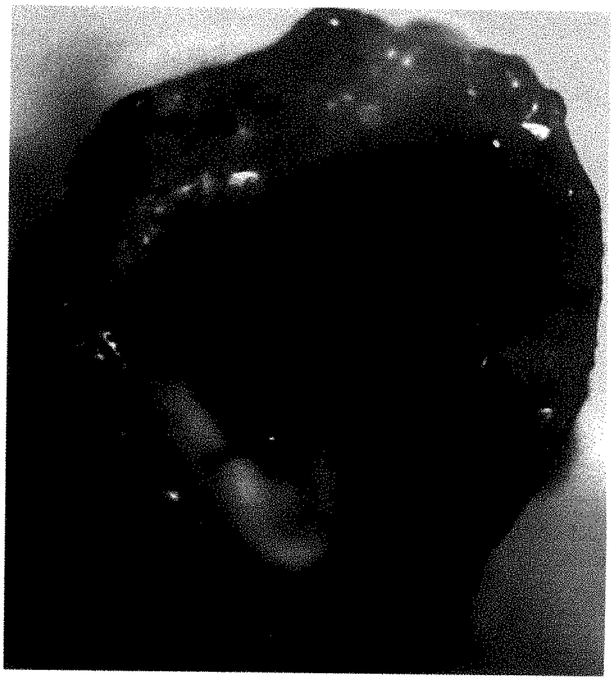

Fig 4. (Patient 11) Laryngeal specimen. Larynx and first tracheal rings were resected after carcinoma recurrence. Gianturco stent is visible, partially covered by inflammatory tissue.

After an average follow-up period of more than 2.5 years and more than 5 years in the older cases, the results achieved confirm our belief in our indications for the use of stents in adults. However, we refuse to extend the indications: for us, the Gianturco prosthesis remains a palliative treatment for tracheal stenoses when external surgical approaches have failed or present too great a risk. Even though the prostheses can be withdrawn, we consider them to be permanent. We do not plan on replacing them, unless a displacement or a migration occurs, which, to date, we have not yet encountered.

Since our first report, the indications for placing the prosthesis have not changed. Table 1, based on our 23 patients, details what we consider to be contraindications for performing surgery via an external approach, hence authorizing the placement of the prosthesis. Those contraindications are refusal of sur-

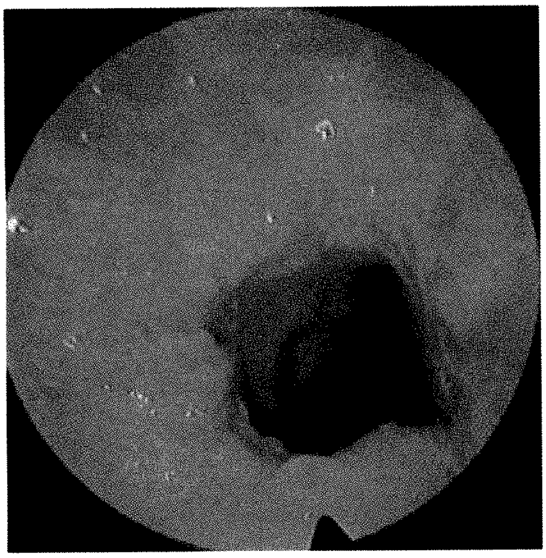

Fig 5. Granuloma formation covering stent (rigid endoscopy).

gery (patient 23); major operative risk because of lack of compliance (patient 1); major cardiopulmonary risks assessed by the resuscitation team; very poor general state (rather than just an age limit); renal insufficiency; diabetes; severe obesity; and extensive tracheal involvement that does not allow a technique of resection and anastomosis or reconstructive surgery with an enlargement technique.

Underlining the temporary nature of the placement, some authors[8] advocate the use of expandable prostheses for young children when a surgical solution based on an external approach is not possible or has failed.

CONCLUSION

Self-expandable prostheses, and the Gianturco prosthesis in particular, can be advocated for long-term treatment of tracheal stenoses in patients with contraindications to an external reconstructive surgical approach. The stents help avoid a permanent tracheotomy. The patient must, however, comply with a strict follow-up in order to prevent the development of granulomas, which represents the most frequent complication.

REFERENCES

1. Colt HG, Dumon JF. Airway stents. Present and future. Clin Chest Med 1995;16:465-78.
2. Dumon JF. A dedicated tracheobronchial stent. Chest 1990;97:328-32.
3. Bolliger CT, Probst R, Tschopp K, Soler M, Perruchoud AP. Silicone stents in the management of inoperable tracheobronchial stenoses. Indications and limitations. Chest 1993;104:1653-9.
4. Nicolai T, Huber RM, Pfeifer KJ, Schneider K, Mantel K, Schott C. Bilateral bronchial balloon dilatation and Strecker stent implantation in a ventilated child with malignant carinal stenosis. Intensive Care Med 1996;22:482-5.
5. Vinograd I, Klin B, Brosch T, Weinberg M, Flomenblit Y, Nevo Z. A new intratracheal stent made from nitinol, an alloy with "shape memory effect." J Thorac Cardiovasc Surg 1994;107:1255-61.
6. Zannini P, Melloni G, Chiesa G, Carretta A. Self-expanding stents in the treatment of tracheobronchial obstruction. Chest 1994;106:86-90.
7. Tsang V, Goldstraw P. Self-expanding metal stent for tracheobronchial strictures. Eur J Cardiothorac Surg 1992;6:555-9.
8. Filler RM, Forte V, Fraga JC, Matute J. The use of expandable metallic airway stents for tracheobronchial obstruction in children. J Pediatr Surg 1995;30:1050-6.
9. Nesbitt JC, Carrasco H. Expandable stents. Chest Surg Clin N Am 1996;6:305-28.
10. Petersen BD, Uchida BT, Barton RE, Keller FS, Rosch J. Gianturco-Rosch Z stents in tracheobronchial stenoses. J Vasc Interv Radiol 1995;6:925-31.
11. Hramiec JE, Haasler GB. Tracheal wire stent complications in malacia: implications of position and design. Ann Thorac Surg 1997;63:209-12.
12. Neustater BR, Goldberg RI. Tracheoesophageal fistula complicating placement of a self-expanding metallic tracheal stent. Am J Gastroenterol 1995;90:1360-1.
13. Nashef SA, Dromer C, Velly JF, Labrousse L, Couraud L. Expanding wire stents in benign tracheobronchial disease: indications and complications. Ann Thorac Surg 1992;54:937-40.
14. Maynar M, Lopez L, Gorriz E, Reyes R, Pulido-Duque JM, Castaneda-Zuniga WR. Massive brachiocephalic artery bleeding due to a Gianturco tracheal stent. J Vasc Interv Radiol 1993;4:289-91.
15. Freitag L, Tekolf E, Stamatis G, Greschuchna D. Clinical evaluation of a new bifurcated dynamic airway stent: a 5-year experience with 135 patients. Thorac Cardiovasc Surg 1997;45:6-12.
16. Tsang V, Williams AM, Goldstraw P. Sequential Silastic and expandable metal stenting for tracheobronchial strictures. Ann Thorac Surg 1992;53:856-60.
17. Remacle M, Lawson G, Minet M, Mayne A, Watelet JB, Jamart J. Endoscopic treatment of tracheal stenosis using the carbon dioxide laser and the Gianturco stent: indications and results. Laryngoscope 1996;106:306-12.
18. Shapshay SM, Beamis JF Jr, Hybels RL, Bohigian RK. Endoscopic treatment of subglottic and tracheal stenosis by radial laser incision and dilation. Ann Otol Rhinol Laryngol 1987;96:661-4.
19. Tan BS, Watkinson AF, Dussek JE, Adam AN. Metallic endoprostheses for malignant tracheobronchial obstruction: initial experience. Cardiovasc Intervent Radiol 1996;19:91-6.
20. Poupart M, Bolot G, Bremond L, Pignat JC. Indications actuelles des prothèses endotrachéales largables type Gianturco (à partie de notre expérience personnelle). Rev Off Soc Franç ORL 1993;7:615-6.
21. Cavaliere S, Venuta F, Foccoli P, Toninelli C, La Face B. Endoscopic treatment of malignant airway obstruction in 2008 patients. Chest 1996;110:1536-42.

22. Kitanosono T, Honda M, Matsui S, et al. Migration of Gianturco expandable metallic stents in the upper trachea. Cardiovasc Intervent Radiol 1997;20:216-8.

23. Rouvière H. Tête et cou. 10th ed. Paris, France: Masson, 1970:501. (Anatomie humaine descriptive et topographique; vol 1.)

24. Colt HG, Harrell J, Neuman TR, Robbins T. External fixation of subglottic tracheal stents. Chest 1994;105:1653-7.

25. Hind CR, Donnelly RJ. Expandable metal stents for tra cheal obstruction: permanent or temporary? A cautionary tale Thorax 1992;47:757-8.

26. Miller RD, Hyatt RE. Evaluation of obstructing lesion of the trachea and larynx by flow-volume curves. Am Rev Respi Dis 1973;108:475-81.

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The XVI Ibero-American Congress of Neurology will be held in Rosario, Argentina, April 16-19, 2000. For information, contac Soledad Piuma, Congresos Internacionales, Moreno 584, 9P, Buenos Aires, Argentina; fax 54 11 3310223; e-mail spiuma@congresosint.com.ar.

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EXPRESSION OF EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEINS LEADS TO CHANGES IN KERATINOCYTE CELL ADHESIO

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Epstein-Barr virus (EBV) has 3 latent membrane proteins (LMPs) — LMP1, LMP2a, and LMP2b — which are expressed nasopharyngeal carcinoma. Using keratinocyte cell lines expressing LMP2a and LMP2b and coexpressing LMP1/LMP2a, we gr organotypic raft cultures to analyze changes in morphology and expression of the cell adhesion molecule ICAM-1; α2, α3, α5, and α6β4 integrins; laminin 5; E-cadherin; and desmoplakin. Cells expressing LMP2a or LMP2b were defective in their ability mature and progress through normal squamous stratification when compared to the parental cell lines. Cells coexpressing LMP LMP2a additionally demonstrated "pseudoinvasion" into the raft dermal equivalent. There was a consistent and dramatic up-regu tion in the suprabasal expression of laminin 5 and α6β4 and β1 integrins in the LMP-expressing cell lines. ICAM-1, not expressed the control cell lines, was up-regulated in the LMP-expressing cell lines. Expression of α3 and α5 integrins was also up-regulated the LMP-expressing cell lines, while α2 demonstrated a loss of the normal basal layer expression. E-cadherin and desmoplak expression patterns were essentially unchanged. We conclude that LMP2a and LMP2b singly, and LMP1/LMP2a coexpressed, capable of altering keratinocyte cell adhesion molecule expression consistent with nasopharyngeal carcinoma.

KEY WORDS — cell adhesion molecules, Epstein-Barr virus, immunocytochemistry, latent membrane proteins.

INTRODUCTION

Epstein-Barr virus (EBV), a double-stranded DNA herpes virus that is the causative agent of infectious mononucleosis, is closely associated with several lymphoproliferative disorders, including nasopharyngeal lymphoma and Burkitt's lymphoma.[1] It is also strongly associated with an epithelial malignancy, nasopharyngeal carcinoma (NPC).[2] The EBV genome includes 3 latent membrane proteins (LMPs): LMP1, LMP2a, and LMP2b.[3,4] In fibroblasts, LMP1 has been found to lead to reduced serum dependence, loss of contact inhibition, anchorage-independent growth, and tumorigenicity in nude mice.[5,6] In B-lymphocytes, LMP1 expression is associated with an increase in cellular aggregation, alterations in the expression of cell adhesion molecules such as intercellular adhesion molecule (ICAM), and changes in cell cycle control.[7-10]

Less is known about LMP2a and LMP2b, which share significant homology. LMP2a interacts with an src-family tyrosine kinase in B-cells, alters cellular calcium regulation, and is involved in the reactivation of lymphocytes from latency.[11-13] The function of LMP2b is unknown.[14]

Much of the work on the effects of LMP protein expression, including studies of cell adhesion molecules, has been performed in lymphocytic cells. Potential LMP effects on cell adhesion molecule expression in epithelial cells are important because of t intimate nature of epithelial cell contacts and the pr gressive maturation patterns found in squamous ep thelium. Our group recently developed several diffe ent LMP-transfected immortalized keratinocyte ce lines: an LMP2a cell line, an LMP2b cell line, and cell line co-expressing LMP1 and LMP2a (LMP LMP2a).[15] To better study the potential effect of LM protein expression on keratinocyte cell morpholo and cell adhesion molecule expression, we employ organotypic raft cell cultures. The advantage of t organotypic culture over a monolayer culture is t ability to evaluate keratinocytes in an interdepende tissuelike system that includes epithelial-dermal su strate interaction and that supports progressive squ mous differentiation. This affords an opportunity compare patterns of expression directly with anal gous in vivo tissues.

MATERIALS AND METHODS

Cell Lines. The human papillomavirus (HPV)–i mortalized human foreskin keratinocyte cell line F 1811 was used as the parent cell line for the LM transfections.[16] These cells were transfected with pLNPOX-based retrovirus vector containing the EB LMP1, LMP2a, or LMP2b genes along with a h gromycin resistance gene (LMP1) or a neomycin r sistance gene (LMP2a, LMP2b) as previously d

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TABLE 1. ANTIBODY SOURCES

Antigen	*Clone*	*Dilution*	*Source*
ICAM-1	10F3	1 μg/mL	Olga Printseva, Washington, DC
α2	P4B4	1:160	William Carter, Fred Hutchinson Cancer Center, Seattle, Wash
α3	P1F2	1:160	William Carter, Fred Hutchinson Cancer Center
α5	P1D6	1:40	William Carter, Fred Hutchinson Cancer Center
β1	8A2	1 μg/mL	Nick Kovack, University of Washington, Seattle, Wash
α6β4	A9	1:5,000	Tom Carey, University of Michigan, Ann Arbor, Mich
Laminin 5	B4-6	1:40	Susana Gill, Fred Hutchinson Cancer Center
Desmoplakin	DP2.15	0.1 μg/mL	Biotech, Rockville, Md
E-cadherin	HECD-1	1 μg/mL	Zymed, San Francisco, Calif

scribed.[15,17] Briefly, PE501 cells were transfected overnight with 10 μg of plasmid DNA with 0.5 mL piperazine-N,N'-bis[2-ethanesulfonic acid]; 1,4-piperazinediethanesulfonic acid buffer, 0.5 mL 0.25-mol/L calcium chloride, and DMEM (Gibco BRL, Gaithersburg, Md)/10% fetal bovine serum (Hyclone, Logan, Utah). One to two milliliters of the PE501 media was filtered through a 0.2-μm filter and placed onto a 30% confluent plate of PG13 cells with 4 μg/mL polybrene in DMEM/10% fetal bovine serum. Selection was performed with 300 U/mL hygromycin (Sigma, St Louis, Mo). Colonies were plated, and 2-mL aliquots of the PG13 colonies' media were filtered through a 0.2-μm filter and added to the FEP 1811 keratinocytes with 4 μg/mL polybrene in keratinocyte serum free media (Gibco BRL). The transfected FEP 1811 keratinocytes were selected with 8 U/mL hygromycin, and expression of the respective latent membrane proteins was demonstrated by Western blot with polyclonal antibodies to LMP2 (gift of Evelyne T. Lennette) and monoclonal antibody CS1-4 to LMP1 (DAKO).[15,18] Additionally, a vector control cell line (FEP 1811-V) was established by using a modification of the pLNPOX plasmid lacking the LMP genes.[17]

Control cell lines (normal human foreskin keratinocytes [HFK], FEP 1811, and FEP 1811-V) and LMP-containing cell lines (LMP2a, LMP2b, and LMP1/LMP2a) were grown in triplicate on organotypic raft cultures as previously described.[19] Briefly, 2 mL of a dermal equivalent consisting of 7 parts purified collagen (Collaborative Biomedical Products, Bedford, Mass), 1 part primary human foreskin fibroblasts, 1 part reconstitution buffer (2.2% $NaCO_3$, 0.05 N NaOH, 200-mmol/L HEPES), and 1 part 10X E media (10 parts DMEM, 3 parts F12; Gibco BRL) were plated onto 35-mm culture dishes and allowed to "set up" at 37°C for 2 hours. Following this step, 1 million keratinocytes in Rheumwald green media were plated on top of the dermal equivalent.[19,20] Two hours later, the entire culture was reamed from its attachment to the plate and submerged in Rheumwald green media. On the sixth day, the entire raft was lifted on a screen and fed at an air-culture interface from below for 2 weeks. The raft cultures were harvested and flash frozen in OCT with liquid nitrogen–cooled isopentane.

Histology and Immunohistochemistry. Specimens were cut on a cryotome into 4-μm sections. Serial sections were stained with hematoxylin and eosin for morphological analysis or reserved for cell adhesion molecule immunohistochemistry that included antibodies to ICAM-1; α2, α3, α5, β1, and α6β4 integrins; laminin 5 (also called epiligrin, kallinin, or nicein); E-cadherin; and desmoplakin. Antibody sources and working dilutions are listed in Table 1. Sections were fixed in acetone for 10 minutes, except for E-cadherin (2% formalin) and desmoplakin (100% ethanol). Following rehydration in phosphate buffered saline (PBS), sections were blocked in 0.3% hydrogen peroxide for 10 minutes and then washed in PBS. The primary antibody was incubated with the tissue sections for 1 hour at room temperature. Following PBS washes, a biotinylated goat anti-mouse secondary antibody (1:500, Vector Labs, Burlingame, Calif) was applied for 30 minutes; the sections were then washed and incubated with avidin biotin complex (Vector Elite) for 30 minutes. The sections were then placed in 0.37 g/mL 3,3' diaminobenzidine/0.06% NiCl/0.17% hydrogen peroxide/0.05-mol/L TRIS for 10 minutes. The slides were washed in distilled water, counterstained with methyl green, and dehydrated in progressive ethanol washes prior to mounting with Histomount. Negative immunohistochemistry controls were run without the primary antibody.

Immunohistochemistry Analysis. Specimens were first analyzed in a qualitative fashion for immunostaining location (eg, basal versus suprabasal). In addition, LMP1/LMP2a immunostaining intensities were compared with LMP2a immunostaining intensities by using scanned photomicrographs and Adobe Photoshop plug-ins on a Macintosh 7200 computer as previously described.[21] Specimens demonstrating an increased intensity of immunostaining greater than a factor of 2 were noted.

TABLE 2. IMMUNOHISTOCHEMISTRY RESULTS

		HFK	*FEP 1811*	*LMP2a*	*LMP2b*	*LMP1/LMP2a*
ICAM-1	SB	−	−	+	+*	++
	B	−	−	+	+	++
$\alpha_6\beta_4$	SB	−	−	+	+	++
	B	+	+	+	+	++
Laminin 5	SB	−	−	+	+	++
	B	+	+	+	+	++
β_1	SB	−	−	+	+	+
	B	+	+	+	+	+
α_3	SB	−	−	+	+	+
	B	+	+	+	+	+
α_5	SB	−	−	+	+	+
	B	+	+	+	+	+
α_2	SB	−	−	+*	+*	+*
	B	+	+	−	−	−
Desmoplakin	SB	+	+	+	+	+
	B	+	+	+	+	+
E-cadherin	SB	+	+	+	+	+
	B	+	+	+	+	+

SB — suprabasal; B — basal; − — no immunostaining; + — positive immunostaining; ++ — intense positive immunostaining (see Materials and Methods). See text for other abbreviations.

*Widely scattered suprabasal cells demonstrating immunostaining.

RESULTS

In all cases the FEP 1811 and FEP 1811-V cell lines behaved similarly, indicating no effect on cell morphology or cell adhesion molecule expression from the retroviral vector alone. Representative results are presented for the FEP 1811 cell type and are summarized in Table 2.

Morphology of Organotypic Cultures. The normal foreskin epithelial cells demonstrate a squamous maturation process that parallels that of in vivo human foreskin (Fig 1A). A well-defined basal layer is clearly seen with a sharp interface between the epithelium and the underlying fibroblast-impregnated collagen matrix. Normal stratification with keratinization is present. The HPV-immortalized FEP 1811 parent cell line (Fig 1B) demonstrates a maturation process that is similar to that of the normal human foreskin epithelial cells. Minor vacuolization of cells can be seen in some areas.

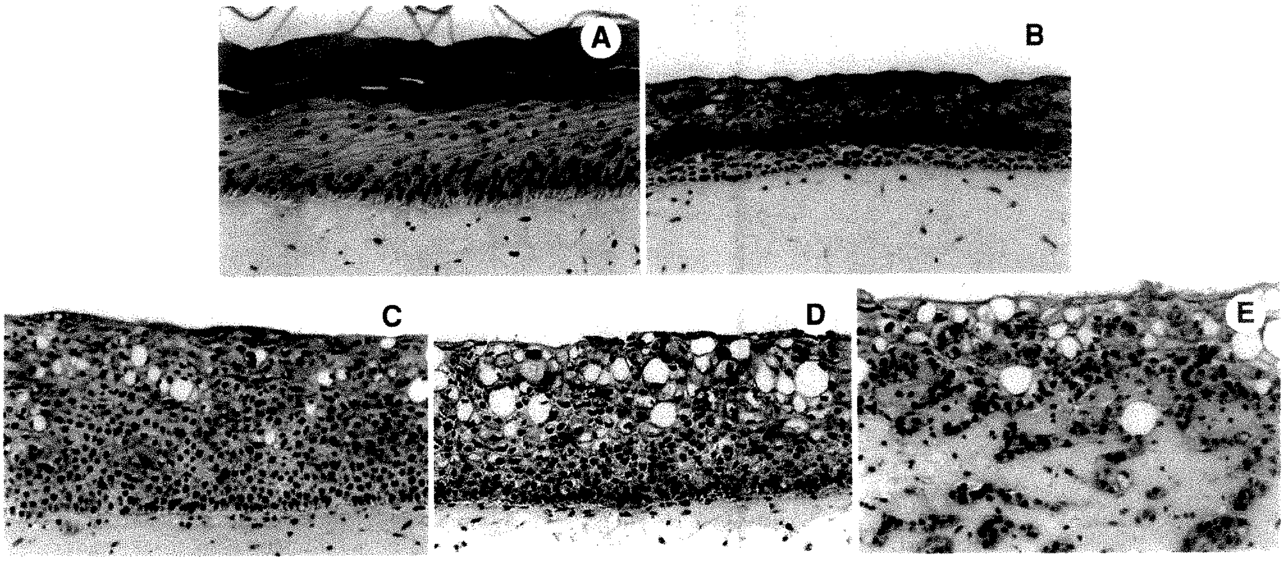

Fig 1. Hematoxylin and eosin–stained sections of organotypic cultures consisting of fibroblast-impregnated collagen matrix and keratinocyte cells on top (original ×200). A) Human foreskin keratinocytes (HFK). B) FEP 1811 cells. C) LMP2a cells. D) LMP2b cells. E) LMP1/LMP2a coexpressing cells. Note sharp epithelial-dermal equivalent interface in all organotypic cultures except for LMP1/LMP2a coexpressors.

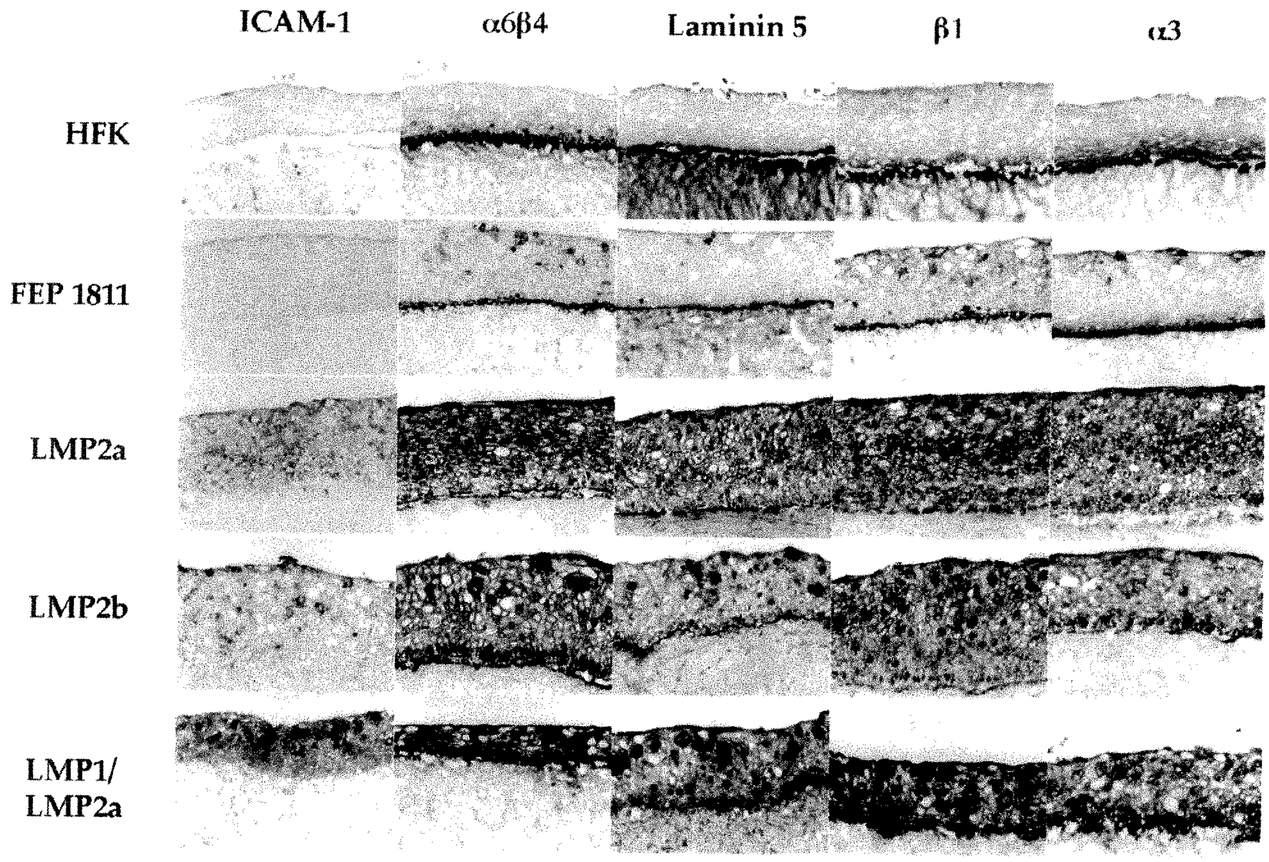

Fig 2. Cell adhesion molecule immunostaining specimens (original ×200). For ease of comparison, immunostained sections are arranged in rows by cell type (HFK, FEP 1811, LMP2a, LMP2b, LMP1/LMP2a) and in columns by antibody type (ICAM-1, α6β4, laminin 5, β1, α3, α5, α2, E-cadherin, desmoplakin). (Continued on next page.)

In organotypic raft cultures made from LMP2a or LMP2b cell lines (Fig 1C,D), a marked increase in the epithelial thickness is found along with an increase in vacuolization compared to the parental FEP 1811 cells. The basal layer remains intact, though it is expanded. While stratification is present in these cells, cell maturation is abnormal, as the flattening of the cell nuclei is delayed. No transition to a squamous morphology is detectable, nor is cornification evident in these sections. The result is an overall increase in apparent disorder throughout the epithelium, with an expansion of the suprabasal layer.

In organotypic raft cultures made from the LMP1/LMP2a coexpressors, a significant change takes place (Fig 1E). There are areas in the rafts in which the basal layer remains intact, along with areas in which the basal layer is replaced by disorganized strings and nests of cells with the morphological characteristics of basal cells, including isolated "pseudoinvasive" islands of cells that are found within the underlying collagen matrix. Where the basal layer remains, the cells are widely spaced and do not align themselves in an orderly manner. Full-thickness change has occurred throughout the epithelium, and no distinct layers are distinguishable. Occasional mitotic figures are found in the superficial aspect of the epithelium.

Cell Adhesion Molecule Immunohistochemistry. In general, the immunostaining patterns seen in the LMP1/LMP2a organotypic rafts did not vary between cells in the "pseudoinvasive" nests and cells in the areas without invasion. For comparison purposes with the other cultures, LMP1/LMP2a figures demonstrate immunostaining in the noninvasive areas.

Expression of ICAM-1 is minimal or nonexistent in the HFK and FEP 1811 parent cell lines (Fig 2, column 1). The LMP2b cells demonstrate scattered cells with clear ICAM-1 expression, while LMP2a cells show a generalized increase in ICAM-1 expression throughout the epithelium. The LMP1/LMP2a coexpressing cells demonstrate a further increase in ICAM-1 immunostaining intensity compared with the LMP2a cell line.

Expression of α6β4 is limited to the most basal layer of keratinocytes in both the HFK and FEP 1811 cell types, with an occasional suprabasal cell demonstrating immunostaining (Fig 2, column 2). The

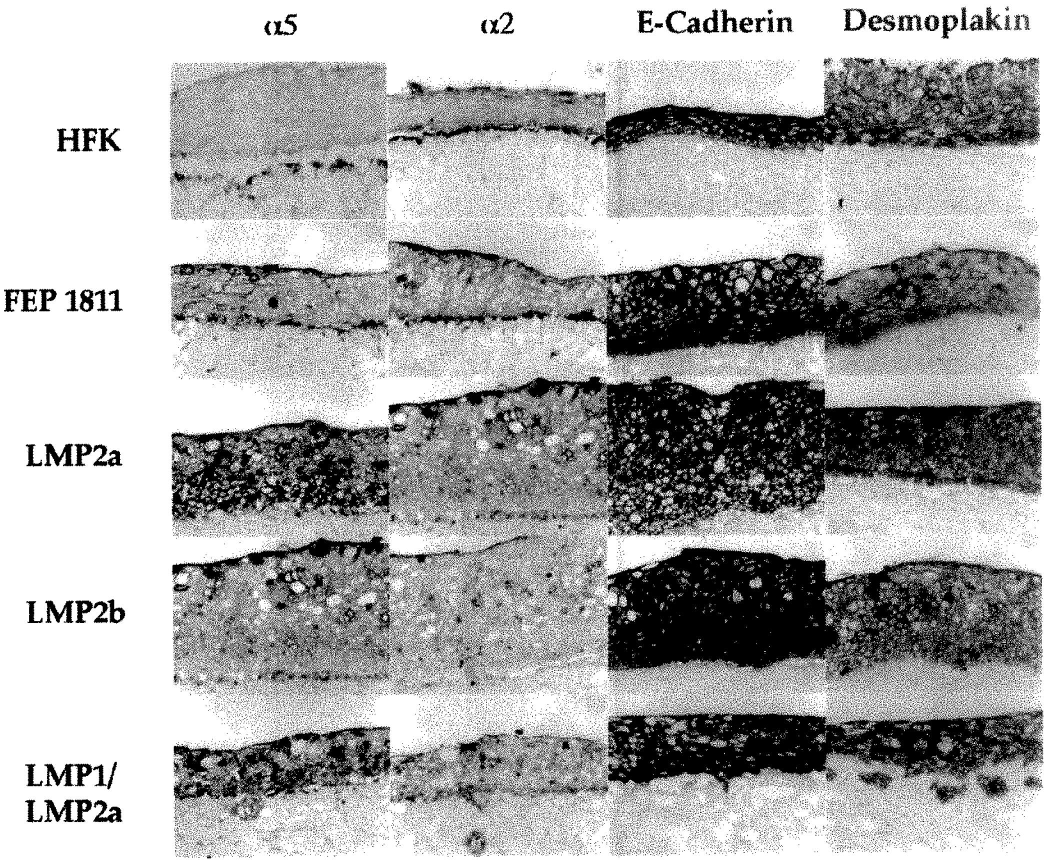

Fig 2, continued.

LMP2a and LMP2b cell lines demonstrate increased staining throughout the epithelium, while in comparison, LMP1/LMP2a coexpressing cells demonstrate a greater level of intense staining throughout the epithelium.

Laminin 5 is restricted to the basal layer in the HFK and FEP 1811 cell lines (Fig 2, column 3). In addition to the basal layer, there is expression throughout the epithelium in the LMP2a and LMP2b cells and more intense staining throughout the LMP1/LMP2a epithelium. Compared with the other cell adhesion molecules, laminin 5 immunostaining also demonstrates a higher background staining in the underlying collagen matrix.

In HFK and FEP 1811 cell lines, β1 integrin expression is primarily limited to the pseudo–basement membrane and the basal layer of keratinocytes (Fig 2, column 4). The intense staining along the pseudo–basement membrane is clearly identified in the HFK organotypic culture, in which there is artifactual separation between the epithelial cell layer and the underlying collagen matrix. In the LMP2a, LMP2b, and LMP1/LMP2a cells, there is increased expression throughout the epithelium.

In HFK and FEP 1811 cell lines, α3 and α5 integrin expression is chiefly limited to the basal keratinocytes (Fig 2, columns 5 and 6). The parent HFK cell line demonstrates a gradually decreasing α3 immunostaining pattern in the immediate suprabasal layers, rather than the sharp suprabasal cutoff seen in the FEP 1811 parent cell line. In the LMP2a, LMP2b, and LMP1/LMP2a cells, there is increased expression throughout the epithelium, with a lesser increase noted for the LMP2b cells.

In contrast to the other integrins, there is a loss of basal layer immunostaining in the LMP-containing cell lines compared to the parent cell lines (Fig 2, column 7).

There is no significant change in E-cadherin expression pattern or immunostaining intensity between the different cell lines (Fig 2, column 8). Similar findings are noted for desmoplakin expression (Fig 2, column 9).

DISCUSSION

Epstein-Barr virus has been strongly linked to NPC of the World Health Organization type II and III classifications, and serology against EBV antigens can be found in a large percentage of NPC patients.[18,22-24] In contrast to EBV infections, which are polyclonal, the EBV genome in EBV-positive NPC is clonal, indicating its presence before malignant transformation, which allows for a possible role in the transformation process.[25] The EBV genome is a linear double-stranded DNA molecule that encodes several proteins, including 6 nuclear proteins and 3 membrane proteins. One of the nuclear proteins, EBV nuclear antigen 1 (EBNA-1), is found in a large percentage of NPCs and is thought to regulate the replication and maintenance of the viral genome during cell proliferation.[26] The 3 EBV membrane proteins are expressed during the latent phase of the infection and are referred to as "latent" membrane proteins: LMP1, LMP2a, and LMP2b. Like EBNA-1, the LMPs are expressed in a large percentage of NPCs. LMP1 is found in up to 60% of NPCs, whereas LMP2a is found in up to 95% of NPCs.[24,26-29] These proteins are thought to play a role in the development and maintenance of the malignant state in the host cell. To date, their mechanisms of action have been investigated primarily in EBV-associated lymphoproliferative disorders such as Burkitt's lymphoma.[4,7,8,13,30-33] While much has been learned about the role of the LMPs in lymphocytes, little is known about their role in the malignant transformation of keratinocytes.

Of the 3 LMP proteins, LMP1 is the best studied. LMP1 is an oncogene encoding a 63 kd transmembrane phosphoprotein with wide-ranging effects on host cells, including the reduction of serum dependence in cultured fibroblasts, changes in cell morphology, and altered cellular adhesion.[34,35] It is known to up-regulate cellular calcium/calmodulin kinases and the bcl-2 oncogene in lymphocytes.[36,37] Also in lymphocytes, it upregulates cyclin D2 expression, increases cdk4/cyclin D2 complexes, and leads to hyperphosphorylation of the retinoblastoma gene product, allowing progression of the cell through the cell cycle.[10] LMP1 has also been shown to increase NF-κB promoter activity, which in turn is linked to the expression of ICAM, IL-8, matrix metalloproteinase 9 (MMP-9), and other cell proteins.[36] LMP1 also affects the differentiation and growth of human and rodent epithelial cells in monolayer culture.[34,35]

Much less is known about the function of the LMP2 family of proteins. LMP2a and LMP2b have significant homology, differing primarily in their 5' exon. LMP2a has been shown to interact with an src-family tyrosine kinase in B-cells and to alter cellular calcium regulation.[11,12] It is involved in a cascade of genetic events that results in the reactivation of lymphocytes from latency.[13] In spite of the fact that the LMP2a protein is found in the vast majority of NPC specimens, its role in keratinocytes and the development of NPC is completely unknown. LMP2b shares a bidirectional promoter with LMP1 and may be functionally linked with LMP1, but as yet has not been assigned a specific function.[14] Neither LMP2a nor LMP2b has been shown to affect cell adhesion molecule expression in the absence of LMP1.

With rare exceptions (eg, occasional suprabasal cells), the parent FEP 1811–immortalized keratinocyte cell line behaved similarly to the nontransformed HFK cell line in expressing cell adhesion molecules and was a useful baseline for comparing the LMP transfectants. Our data demonstrate profound differences both in keratinocyte morphology and cell adhesion phenotypes for the LMP2a, LMP2b, and LMP1/LMP2a transfected cell lines when compared to the FEP 1811 parent cell line. LMP2a alone was associated with qualitative changes in cell adhesion molecule expression, while the addition of LMP1 in the LMP1/LMP2a transfectants generally resulted only in further quantitative changes in cell adhesion marker expression. In normal squamous epithelium, expression of the majority of the cell adhesion molecule panel we evaluated ($\alpha2$, $\alpha3$, $\alpha5$, $\alpha6\beta4$, $\beta1$, laminin 5) is limited to the basal cell layer in which the proliferating "stem cell" population of the epithelium resides.[38-43] In the HFK and FEP 1811 control organotypic cultures, this basal layer pattern of expression persists. In contrast, LMP2a- and LMP2b-transfected cells demonstrate suprabasal expression of most of these cell adhesion molecules. In the case of $\alpha2$, there was a loss of expression in the basal layer matching the lack of expression in the suprabasal layers, while conversely, there was a gain of ICAM-1 expression throughout the epithelium where there had been none before. LMP1/LMP2a coexpressors demonstrated the same intraepithelial pattern changes as the LMP2a cells, but also exhibited more intense immunostaining. These changes in cell adhesion molecule expression patterns are the same as those seen in head and neck premalignancies and carcinomas in vivo.[44-46] As the basal cell epithelial population differentiates, integrin expression is generally lost, while conversely, the retention of integrin expression is associated with retention of the basal cell phenotype.[47]

We cannot currently study the effect of LMP1 alone in the organotypic system for lack of a stable LMP1-only clone, but LMP1 lymphocyte experiments suggest several possible mechanisms for LMP interactions with cell adhesion molecule expression. As previously mentioned, changes in NF-κB activity have been linked to increased expression of ICAM. In addition, there are data to suggest that LMP1 acts as a constitutively signaling TNF-α receptor–like molecule.[33] In addition to up-regulating NF-κB, TNF-α can increase the expression of ICAM and β1, α1, α2, and α6 integrins.[48-52] While it has been demonstrated that some cell types immortalized with the HPV 16 genome have constitutively up-regulated TNF-α, which might up-regulate ICAM-1 expression, we do not see such an effect with the HPV 18–immortalized FEP 1811 cell line.[53] With the introduction of the LMP genes, however, we see a dramatic change in ICAM-1 expression. The increased expression of ICAM is not a universal phenomenon associated with EBV transfection in vivo. While ICAM expression is down-regulated in EBV-positive Burkitt's lymphoma cell lines, it is frequently up-regulated in NPC.[44,45,54] Again, the organotypic cultures parallel the in vivo findings.

Lymphocytic infiltration in NPC is a common finding, and it has been suggested that the lymphocytes may regulate the growth of NPC tumor cells via a paracrine mechanism, or alternatively, that they may be part of an antitumor immune response.[55] ICAM expression may play a complex role in the NPC phenotype, since ICAM is targeted by the lymphocyte LFA-1 receptor.[45,56] Lymphocytic infiltration in NPC has been associated with improved prognosis.[57] Conversely, others have demonstrated that LMP1 expression is frequently lost in poorly differentiated NPC, which has a worse prognosis.[24] The link between these observations may be ICAM expression by the NPC cells driven by LMP protein expression.

No previous descriptions of LMP effects on α6β4 expression have been published. However, the dramatic changes in the expression of α6β4 in the LMP organotypic cultures are in strong agreement with the changes seen in squamous dysplasia and carcinoma in vivo.[46,58-60] Further, it has been suggested that laminin 5 and α6β4 are coregulated.[61] Our data fit with this hypothesis, since α6β4 and its ligand laminin 5 are coexpressed in identical fashion in the LMP-transfected organotypic cultures.

The morphological changes seen in the LMP organotypic cultures were consistent with the alterations seen in cell adhesion molecule expression. In the LMP2a and LMP2b cultures, a marked increase in the epithelial thickness is found, along with an expanded basal layer. Some stratification is present in the suprabasal layers, but the overall cell maturation is abnormal, with an apparent increase in disorder throughout the epithelium. Compared with the LMP2a cultures, the coexpressing LMP1/LMP2a cultures are more compact, but demonstrate an increase in cellularity with a loss of maturation when compared with the parent FEP 1811 cell line. Further, the LMP1/LMP2a cell line consistently demonstrated a "pseudoinvasive" component that violated the epithelial–collagen matrix interface. This phenotype is referred to as pseudoinvasive because a true basement membrane does not exist in the organotypic cultures, though there is evidence for a basal membrane–oriented deposition of some materials (eg, β1 expression). As would be expected, deliberate manipulation of the collagen matrix firmness can influence the relative amount of "pseudoinvasion" and can result in "pseudoinvasion" in organotypic cell cultures made from the other cell types. But the fact remains that the LMP1/LMP2a coexpressors consistently demonstrated "pseudoinvasion" under conditions in which the other cell lines did not. This consistent finding raises the possibility that another change associated with LMP1 expression might be taking advantage of the changes in cell adhesion molecule expression. One possible LMP1-inducible cofactor is the metalloproteinase MMP-9, which is associated with NF-κB promoter activity.[36] Further studies of this issue will require separating the effect of LMP1 from that of LMP2a in the keratinocytes.

REFERENCES

1. Young L, Alfieri C, Hennessy K, et al. Expression of Epstein-Barr virus transformation-associated genes in tissues of patients with EBV lymphoproliferative disease. N Engl J Med 1989;321:1080-5.

2. Liebowitz D. Nasopharyngeal carcinoma: the Epstein-Barr virus association. Semin Oncol 1994;21:376-81.

3. Hennessy K, Fennewald S, Hummel M, Cole T, Kieff E. A membrane protein encoded by Epstein-Barr virus in latent growth-transforming infection. Proc Natl Acad Sci U S A 1984; 81:7207-11.

4. Longnecker R, Kieff E. A second Epstein-Barr virus membrane protein (LMP2) is expressed in latent infection and colocalizes with LMP1. J Virol 1990;64:2319-26.

5. Wang D, Liebowitz D, Kieff E. An EBV membrane protein expressed in immortalized lymphocytes transforms established rodent cells. Cell 1985;43:831-40.

6. Baichwal VR, Sugden B. Transformation of Balb 3T3 cells by the BNLF-1 gene of Epstein-Barr virus. Oncogene 1988;2:461-7.

7. Peng M, Lundgren E. Transient expression of the Epstein-Barr virus LMP1 gene in human primary B cells induces cellular activation and DNA synthesis. Oncogene 1992;7:1775-82.

8. Wang F, Gregory C, Sample C, et al. Epstein-Barr virus latent membrane protein (LMP1) and nuclear proteins 2 and 3C are effectors of phenotypic changes in B lymphocytes: EBNA-2 and LMP1 cooperatively induce CD23. J Virol 1990;64:2309-18.

9. Wang F, Tsang SF, Kurilla MG, Cohen JI, Kieff E. Epstein-Barr virus nuclear antigen 2 transactivates latent membrane protein LMP1. J Virol 1990;64:3407-16.

10. Arvanitakis L, Yaseen N, Sharma S. Latent membrane protein-1 induces cyclin D2 expression, pRb hyperphosphory-

lation, and loss of TGF–beta 1–mediated growth inhibition in EBV-positive B cells. J Immunol 1995;155:1047-56.

11. Longnecker R. Biochemical and genetic studies of Epstein-Barr virus latent membrane protein 2. Leukemia 1994;1: S46-S50.

12. Longnecker R, Druker B, Roberts TM, Kieff E. An Epstein-Barr virus protein associated with cell growth transformation interacts with a tyrosine kinase. J Virol 1991;65:3681-92.

13. Miller CL, Lee JH, Kieff E, Burkhardt AL, Bolen JB, Longnecker R. Epstein-Barr virus protein LMP2A regulates reactivation from latency by negatively regulating tyrosine kinases involved in sIg-mediated signal transduction. Infect Agents Dis 1994;3:128-36.

14. Laux G, Economou A, Farrell PJ. The terminal protein gene 2 of Epstein-Barr virus is transcribed from a bidirectional latent promoter region. J Gen Virol 1989;70:3079-84.

15. Winberg G, McDougall JK, Coltrera MD. Expression of Epstein-Barr virus membrane proteins LMP1, LMP2a, and LMP2b in human keratinocytes. Assoc Res Otolaryngol 1996; 19:52.

16. Kaur P, McDougall JK. HPV-18 immortalization of human keratinocytes. Virology 1989;173:302-10.

17. Miller AD, Rosman GJ. Improved retroviral vectors for gene transfer and expression. Biotechniques 1989;7:980-2.

18. Lennette ET, Winberg G, Yadav M, Enblad G, Klein G. Antibodies to LMP2A/2B in EBV-carrying malignancies. Eur J Cancer 1995;11:1875-8.

19. Blanton RA, Perez Reyes N, Merrick DT, McDougall JK. Epithelial cells immortalized by human papillomaviruses have premalignant characteristics in organotypic culture. Am J Pathol 1991;138:673-85.

20. Asselineau D, Bernhard B, Bailly C, Darmon M. Epidermal morphogenesis and induction of the 67 kd keratin polypeptide by culture of human keratinocytes at the liquid-air interface. Exp Cell Res 1985;159:536-9.

21. Lehr HA, Hansen KD, Coltrera MD, Russ JC, Gown AM. Photoshop-based image analysis for the semi-automated assessment of Ki-67–defined proliferative activity in the routine diagnosis of breast cancer. Appl Immunohistochem 1996;4:117-27.

22. Frech B, Zimber Strobl U, Suentzenich KO, et al. Identification of Epstein-Barr virus terminal protein 1 (TP1) in extracts of four lymphoid cell lines, expression in insect cells, and detection of antibodies in human sera. J Virol 1990;64:2759-67.

23. Frech B, Zimber Strobl U, Yip TT, Lau WH, Mueller Lantzsch N. Characterization of the antibody response to the latent infection terminal proteins of Epstein-Barr virus in patients with nasopharyngeal carcinoma. J Gen Virol 1993;74:811-8.

24. Niedobitek G, Young LS, Sam CK, Brooks L, Prasad U, Rickinson AB. Expression of Epstein-Barr virus genes and of lymphocyte activation molecules in undifferentiated nasopharyngeal carcinomas. Am J Pathol 1992;140:879-87.

25. Raab Traub N, Flynn K. The structure of the termini of the Epstein-Barr virus as a marker of clonal cellular proliferation. Cell 1986;47:883-9.

26. Brooks L, Yao QY, Rickinson AB, Young LS. Epstein-Barr virus latent gene transcription in nasopharyngeal carcinoma cells: coexpression of EBNA1, LMP1, and LMP2 transcripts. J Virol 1992;66:2689-97.

27. Fahraeus R, Fu HL, Ernberg I, et al. Expression of Epstein-Barr virus–encoded proteins in nasopharyngeal carcinoma. Int J Cancer 1988;42:329-38.

28. Young LS, Dawson CW, Clark D, et al. Epstein-Barr virus gene expression in nasopharyngeal carcinoma. J Gen Virol 1988;69:1051-65.

29. Busson P, McCoy R, Sadler R, Gilligan K, Tursz T, Raab Traub N. Consistent transcription of the Epstein-Barr virus LMP2 gene in nasopharyngeal carcinoma. J Virol 1992;66:3257-62.

30. Longnecker R, Miller CL, Miao XQ, Tomkinson B, Kieff E. The last seven transmembrane and carboxy-terminal cytoplasmic domains of Epstein-Barr virus latent membrane protein 2 (LMP2) are dispensable for lymphocyte infection and growth transformation in vitro. J Virol 1993;67:2006-13.

31. Kaye KM, Izumi KM, Kieff E. Epstein-Barr virus latent membrane protein 1 is essential for B-lymphocyte growth transformation. Proc Natl Acad Sci U S A 1993;90:9150-4.

32. Salcedo R, Fuerstenberg SM, Patarroyo M, Winberg G. The Epstein-Barr virus BNLF-1 membrane protein LMP1 induces homotypic adhesion mediated by CD11a/CD18 in a murine B-cell line, mimicking the action of phorbol ester. J Virol 1991;65:5558-63.

33. Mosialos G, Birkenbach M, Yalamanchili R, VanArsdale T, Ware C, Kieff E. The Epstein-Barr virus transforming protein LMP1 engages signaling proteins for the tumor necrosis factor receptor family. Cell 1995;80:389-99.

34. Fahraeus R, Rymo L, Rhim JS, Klein G. Morphological transformation of human keratinocytes expressing the LMP gene of Epstein-Barr virus. Nature 1990;345:447-9.

35. Dawson CW, Rickinson AB, Young LS. Epstein-Barr virus latent membrane protein inhibits human epithelial cell differentiation. Nature 1990;344:777-80.

36. Rowe M, Peng Pilon M, Huen DS, et al. Upregulation of bcl-2 by the Epstein-Barr virus latent membrane protein LMP1: a B-cell–specific response that is delayed relative to NF-kappa B activation and to induction of cell surface markers. J Virol 1994;68:5602-12.

37. Mosialos G, Hanissian SH, Jawahar S, Vara L, Kieff E, Chatila TA. A Ca2+/calmodulin-dependent protein kinase, CaM kinase–Gr, expressed after transformation of primary human B lymphocytes by Epstein-Barr virus (EBV) is induced by the EBV oncogene LMP1. J Virol 1994;68:1697-705.

38. Wayner EA, Carter WG. Identification of multiple cell adhesion receptors for collagen and fibronectin in human fibrosarcoma cells possessing unique alpha and common beta subunits. J Cell Biol 1987;105:1873-84.

39. Peltonen J, Larjava H, Jaakkola S, et al. Localization of integrin receptors for fibronectin, collagen, and laminin in human skin. Variable expression in basal and squamous cell carcinomas. J Clin Invest 1989;84:1916-23.

40. Carter WG, Ryan MC, Gahr PJ. Epiligrin, a new cell adhesion ligand for integrin alpha 3 beta 1 in epithelial basement membranes. Cell 1991;65:599-610.

41. Carter WG, Wayner EA, Bouchard TS, Kaur P. The role of integrins alpha 2 beta 1 and alpha 3 beta 1 in cell-cell and cell-substrate adhesion of human epidermal cells. J Cell Biol 1990;110:1387-404.

42. Hodivala KJ, Watt FM. Evidence that cadherins play a role in the downregulation of integrin expression that occurs during keratinocyte terminal differentiation. J Cell Biol 1994; 124:589-600.

43. Van Waes C, Surh DM, Chen Z, et al. Increase in suprabasilar integrin adhesion molecule expression in human epidermal neoplasms accompanies increased proliferation occurring with immortalization and tumor progression. Cancer Res 1995; 55:5434-44.

44. Ruco LP, Stoppacciaro A, Uccini S, et al. Expression of intercellular adhesion molecule–1 and vascular cell adhesion molecule–1 in undifferentiated nasopharyngeal carcinoma (lymphoepithelioma) and in malignant epithelial tumors. Hum Pathol 1994;25:924-8.

45. Busson P, Zhang Q, Guillon JM, et al. Elevated expression of ICAM1 (CD54) and minimal expression of LFA3 (CD58) in Epstein-Barr–virus–positive nasopharyngeal carcinoma cells. Int J Cancer 1992;50:863-7.

46. Van Waes C, Kozarsky KF, Warren AB, et al. The A9 antigen associated with aggressive human squamous carcinoma is structurally and functionally similar to the newly defined integrin alpha 6 beta 4. Cancer Res 1991;51:2395-402.

47. Jones J, Sugiyama M, Watt FM, Speight PM. Integrin expression in normal, hyperplastic, dysplastic, and malignant oral epithelium. J Pathol 1993;169:235-43.

48. Voraberger G, Schafer R, Stratowa C. Cloning of the human gene for intercellular adhesion molecule 1 and analysis of its 5'-regulatory region. Induction by cytokines and phorbol ester. J Immunol 1991;147:2777-86.

49. van Kooten C, Rensink I, Aarden L, van Oers R. TNF-alpha induces spreading of B-CLL via the CD11c/CD18 molecule. Am J Hematol 1993;44:221-8.

50. Felch ME, Willis RA, Penney DP, Keng PC, Phipps RP. Expression of alpha 6 beta 1 integrin, the laminin receptor, on subsets of normal murine lung fibroblasts and its upregulation by the inflammatory cytokines IFN-gamma and TNF-alpha. Reg Immunol 1992;4:363-70.

51. Santala P, Heino J. Regulation of integrin-type cell adhesion receptors by cytokines. J Biol Chem 1991;266:23505-9.

52. Ioffreda MD, Whitaker D, Murphy GF. Mast cell degranulation upregulates alpha 6 integrins on epidermal Langerhans cells. J Invest Dermatol 1993;101:150-4.

53. Malejczyk J, Malejczyk M, Kock A, et al. Autocrine growth limitation of human papillomavirus type 16–harboring keratinocytes by constitutively released tumor necrosis factor–alpha. J Immunol 1992;149:2702-8.

54. Gregory CD, Murray RJ, Edwards CF, Rickinson AB. Downregulation of cell adhesion molecules LFA-3 and ICAM-1 in Epstein-Barr virus–positive Burkitt's lymphoma underlies tumor cell escape from virus-specific T cell surveillance. J Exp Med 1988;167:1811-24.

55. Zong YS, Lin H, Choy DT, et al. Nasopharyngeal carcinoma and lymphoinfiltration. Oncology 1991;48:290-6.

56. Peng M, Lundgren E. Transient expression of the Epstein-Barr virus LMP1 gene in B-cell chronic lymphocytic leukemia cells, T cells, and hematopoietic cell lines: cell-type–independent induction of CD23, CD21, and ICAM-1. Leukemia 1993; 7:104-12.

57. Fandi A, Altun M, Azli N, Armand JP, Cvitkovic E. Nasopharyngeal cancer: epidemiology, staging, and treatment. Semin Oncol 1994;21:382-97.

58. Wolf GT, Carey TE. Tumor antigen phenotype, biologic staging, and prognosis in head and neck squamous carcinoma. Monogr Natl Cancer Inst 1992;13:67-74.

59. Van Waes C, Carey TE. Overexpression of the A9 antigen/alpha 6 beta 4 integrin in head and neck cancer. Otolaryngol Clin North Am 1992;25:1117-39.

60. Liebert M, Washington R, Wedemeyer G, Carey TE, Grossman HB. Loss of co-localization of alpha 6 beta 4 integrin and collagen VII in bladder cancer. Am J Pathol 1994;144:787-95.

61. Savoia P, Trusolino L, Pepino E, Cremona O, Marchisio PC. Expression and topography of integrins and basement membrane proteins in epidermal carcinomas: basal but not squamous cell carcinomas display loss of alpha 6 beta 4 and BM-600/nicein. J Invest Dermatol 1993;101:352-8.

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DISTANT LYMPHATIC METASTASIS FROM HEAD AND NECK CANCER

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A predictable pattern of metastasis based on tumor histology and site of origin has been well documented for most cancers that arise in the head and neck region. The current study demonstrates that this predictable pattern of metastasis can be significantly impacted by previous therapy, resulting in unusual patterns of metastasis in patients with recurrent tumors. A retrospective case series of 5 patients with head and neck carcinomas who developed metastases to distant lymph nodes is presented. All patients underwent surgery and radiotherapy to the primary tumor and regional lymphatics at the time of their initial treatment. All of the patients developed a local recurrence less than a year before the detection of distant lymphatic metastases. Cytology or excision confirmed metastases to the axillary, inguinal, or anterior intercostal lymph nodes. All of the patients underwent aggressive surgery for attempted cure of the local recurrence shortly before the presence of distant lymphatic metastases was clinically recognized. The metastatic workup of patients with carcinomas of the head and neck frequently includes examination of the regional lymph nodes as well as chest radiography, liver function tests, and serum calcium determination. This evaluation may fail to detect metastases to distant lymph nodes in patients who present with recurrent or second primary cancers. Such patients should undergo careful examination of all major lymph node–bearing regions of the body when being evaluated for the presence of distant metastases.

KEY WORDS — head and neck cancer, lymph node, metastasis.

INTRODUCTION

As local and regional control of head and neck cancer has improved, distant metastases have become an increasingly common cause of death.[1] In 1923, Crile[2] reported that only 1% of patients with cancer of the upper aerodigestive tract had distant metastasis at autopsy. Some 40 years later, studies of postmortem examinations conducted after the refinement of techniques for the treatment of head and neck cancer by surgery and radiotherapy revealed distant metastases in 36% to 57% of cases.[3,4] For squamous cell carcinoma of the upper aerodigestive tract, the most common metastasis is to the cervical regional lymph nodes. The lungs, liver, and bones are the most common sites of distant hematogenous metastases.[4,5] Other, less common sites of metastases include the mediastinum, adrenal gland, brain, pericardium, kidney, and thyroid gland.[6]

The disturbance of the lymphatic system in the cervical region resulting from radiotherapy or neck dissection can result in alternative pathways of lymphatic drainage. These newly formed pathways of drainage can ultimately result in lymphatic dissemination of head and neck cancers to sites below the clavicles. Metastasis from head and neck carcinomas to infraclavicular lymph nodes has been reported very infrequently in the literature.[7,8] Recognition of this phenomenon is crucial in the evaluation of patients with recurrent head and neck cancer, especially when salvage surgery is entertained. Screening tests for distant metastases that are commonly performed in these patients, including chest radiography, liver function tests, and determination of a serum calcium level, may fail to detect metastases to infraclavicular lymph nodes.

MATERIALS AND METHODS

The University of California Los Angeles Medical Center Tumor Registry was reviewed to identify patients evaluated for mucosal squamous cell carcinoma during a 2-year interval. A detailed chart review was performed for 5 patients treated for metastases to infraclavicular lymph nodes.

RESULTS

Review of the tumor registry revealed that 342 patients were evaluated at University of California Los Angeles School of Medicine between July 1995 and June 1997 for mucosal head and neck squamous cell carcinoma. Forty-seven (13.7%) of these cases were found to have distant metastasis. Five patients (1.5%) were found to have metastases to infraclavic-

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TABLE 1. CLINICAL PROFILE AND TREATMENT AT INITIAL PRESENTATION

Case	Age (y)	Primary Site	TNM	Treatment	Neck Dissection	XRT (Gy)
1	62	Right floor of mouth	T3N0M0	Composite resection	Bilateral	61
2	61	Hypopharyngeal	T3N2M0	Total laryngectomy	Bilateral	70
3	72	Right retromolar	T2N1M0	Composite resection	Right	60
4	60	Left retromolar	T2N0M0	Composite resection	Left	70
5	70	Left tonsil	T4N0M0	Composite resection	Bilateral	72

ular lymph nodes. These patients' ages ranged from 60 to 72 years (mean 65 years, median 62 years). There were 3 women and 2 men. Distant nodal metastasis to infraclavicular lymph nodes was detected and confirmed by cytology or histology of an excised lymph node in all cases.

Patient characteristics and treatment summaries of the 5 patients treated for distant lymphatic metastases are summarized in Tables 1 and 2. All patients previously had been treated for primary mucosal squamous cell carcinomas of the head and neck region. The initial treatment for all patients entailed surgical resection of the primary lesion along with neck dissection. An ipsilateral neck dissection was performed on 2 patients, while bilateral neck dissections were performed on 3 patients. All of the patients also received postoperative radiotherapy to the primary site and both sides of the neck. The average dose of radiotherapy was 66.6 Gy (median 70 Gy).

Recurrent aerodigestive mucosal carcinomas occurred in all 5 patients less than 2 years before the clinical recognition of distant lymphatic metastases. This included 3 patients with recurrence at the previously treated primary tumor site, and 2 patients with secondary primary carcinomas of the head and neck. A head and neck and general physical examination, a chest radiograph, and blood tests consisting of liver function tests and a serum calcium level determination were performed on all of the patients. No distant metastases were detected in any of the 5 patients by this evaluation. One patient was determined to be unsalvageable because of skull base involvement by a recurrent carcinoma of the tonsil and infratemporal fossa (case 5). The remaining 4 patients underwent aggressive resection and flap reconstruction to treat their recurrent primary carcinomas. Pathological examination of the excised specimens confirmed all of the margins to be free of tumor in these 4 patients who underwent salvage surgery.

TABLE 2. PROFILE OF PATIENTS WITH RECURRENCE AND DISTANT NODAL METASTASES

Case	Recurrence	Salvage Surgery	Site of Nodal Metastasis	Postrecurrence Metastases (mo)*
1	R floor of mouth	Yes	Presternal	11
2	R base of tongue	Yes	L inguinal	4
3	R floor of mouth	Yes	L axillary	4
4	R floor of mouth	Yes	R axillary	23
5	L infratemporal	No	Presternal	3

*Time between diagnosis of primary tumor recurrence and distant nodal metastases.

Distant lymphatic metastasis was clinically recognized between 3 months and 23 months (median 4 months) after the diagnosis of upper aerodigestive tract recurrences or second primaries. All patients developed distant lymphatic metastases that demonstrated pathological features that were consistent with the previously treated head and neck primaries. Three cases were confirmed by excision of the distant lymphatic metastases, while 2 cases were demonstrated by fine needle aspiration cytology. The sites of these distant lymphatic metastases included the axillary lymphatic chain in 2 cases, anterior intercostal lymph nodes in 2 cases, and inguinal lymph nodes in 1 case.

Further evaluation for other sites of tumor recurrence was performed on all of the patients upon the diagnosis of distant lymphatic metastasis. This workup included physical examinations, chest and abdominal computed tomography scans, bone scans, and serum chemistry studies. The results showed no other sites of distant metastasis in any of the patients. Treatment of the distant lymphatic metastases included regional lymphadenectomy and radiotherapy in 2 cases, local radiotherapy alone in 2 cases, and local radiotherapy combined with chemotherapy in 1 case. Only 1 of the patients has remained free of disease for 1 year, after an axillary lymph node dissection and radiotherapy (case 3). The remainder have either died or developed multifocal distant metastasis.

DISCUSSION

It is generally agreed that head and neck cancer remains confined above the clavicles in the majority of cases. A clinical series that included 5,019 patients with head and neck cancer identified an 11% incidence of distant metastasis.[9] However, autopsy studies of patients with head and neck cancer have demonstrated the incidence of distant metastasis to be as high as 57%.[4] Once distant metastases are detected,

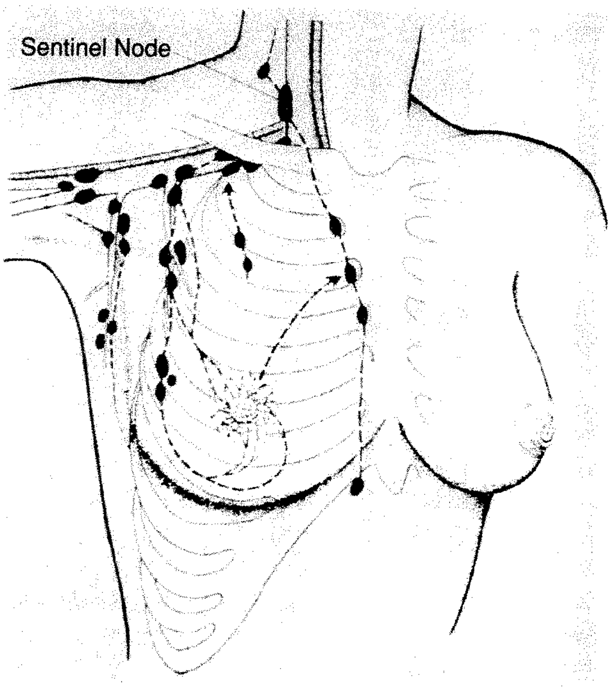

Fig 1. Lymphatic connection between subclavicular pathways (axillary, internal mammary) and supraclavicular sentinel node.[14] (Reprinted with permission from Clouse ME. Lymphatic imaging: lymphography, computer tomography and scintigraphy. 2nd ed. Baltimore, Md: Williams & Wilkins, 1985:398.)

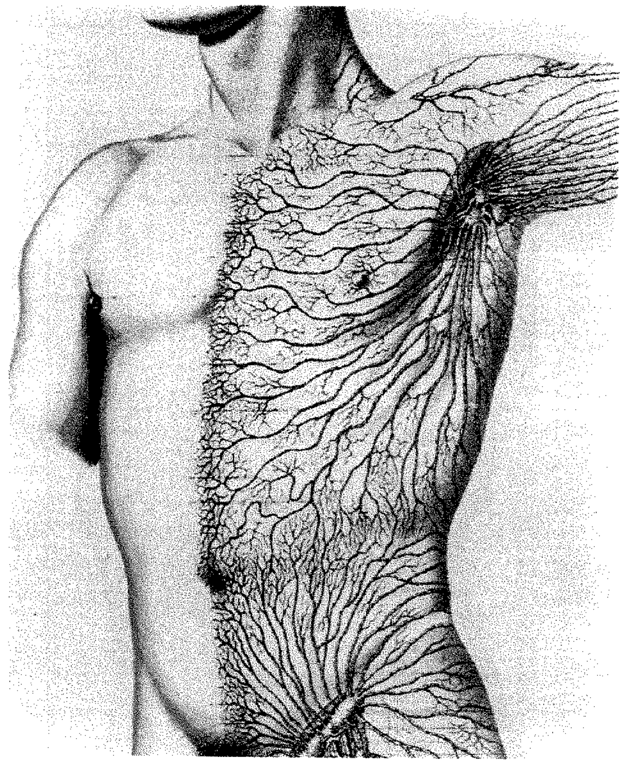

Fig 2. Mercurial injection of lymphatic system, showing array of lymphatic anastomoses extending from cervical region to inguinal region. (Reprinted with permission.[16])

patients have a very poor prognosis. The time interval between the diagnosis of distant metastasis and death is less than 2 years in greater than 90% of such cases.[10] At our institution during this study period, the prevalence of distant metastasis among patients with mucosal squamous cell carcinoma of the head and neck was 14%, with 96% of such cases surviving less than 2 years.

A correlation between the incidence of regional metastases and distant metastases has been well shown for head and neck cancer. Distant metastases are also more common after recurrence of local or regional disease. Leibel and Scott[11]and Calhoun et al[12] have reported that two thirds of the patients with distant metastases had recurrent locoregional carcinoma. In the current series, local recurrence or secondary primary carcinomas were detected in all 5 patients less than 2 years before the recognition of distant lymphatic metastases.

A predictable pattern of lymphatic metastasis based on tumor histology and site of origin has also been well documented for most cancers that arise in the head and neck region. However, previous surgery or radiotherapy can significantly alter this predictable pattern of lymphatic tumor dissemination. A neck dissection removes lymph nodes and causes physical disruption of lymphatic channels in the cervical region. Radiotherapy produces sclerosis of lymph nodes and fibrosis of lymphatic vessels, which results in obstructive lymphedema.[13] Therefore, both radiotherapy and neck dissection can result in the eradication of the primary lymphatics of the head and neck region. The lymphatic stasis that subsequently develops is relieved by the development of collateral channels through alternative drainage pathways. These pathways do not follow a predicted pattern of drainage, but may form anastomoses with lymphatic channels in the infraclavicular region.

These alternative pathways have been demonstrated in previous anatomic studies of the human lymphatic system. In 1932, Rouviere[14] reported the retrograde flow of lymph fluid through lymphatics from the superomedial part of the breast and axilla to the deep sentinel nodes, near the jugular-subclavian venous confluence (Fig 1). These sentinel nodes are in direct communication with the transverse cervical lymph nodes within the supraclavicular fossa. Both Rouviere[14] and Bartels[15] identified lymphatic channels coursing from the axillary lymph nodes to the transverse cervical lymphatic chain. Reverse flow from the supraclavicular, transverse cervical, and spinal accessory lymphatic chains into the central axillary nodes has also been shown.[16] These studies show that bidirectional lymph flow occurs across the clavicles through multiple channels. The presence of these pathways was also illustrated by Sappey.[17]

Once the lymph flow reaches the infraclavicular region, there are 3 lymphatic channels on each side of the thoracic cavity, which can distribute its contents to a variety of locations. The paired internal thoracic trunks course along both sides of the sternum and communicate with the parasternal and anterior intercostal lymph nodes. The left-sided thoracic duct and right lymph trunk are located adjacent to the vertebrae and provide drainage for the chest wall. The mediastinal lymph trunks course along the trachea and drain the lungs, esophagus, paratracheal region, and heart. These 3 pairs of lymph trunks communicate freely with each other. There are also anastomoses between these groups of lymphatics and the axillary nodal chain as well as the internal thoracic trunk in the hypoepigastric region. Such an array of lymphatic anastomoses, from the supraclavicular region to the infraclavicular region, makes it possible for a head and neck carcinoma to manifest itself in any lymphatic region below the clavicle (Fig 2[16]). This possibility may account for the occurrence of parasternal, inguinal, and axillary nodal metastases in the current series. All of the patients in the current study had treatment consisting of surgery and radiotherapy that obliterated bilateral cervical lymphatics, thereby opening collateral lymphatic channels to ipsilateral and contralateral axillary, anterior intercostal, and inguinal lymph nodes.

The optimal management of patients with distant lymphatic metastases remains poorly defined because of the low incidence of occurrence. The prognosis of patients with distant lymphatic metastases from head and neck cancer may be better than that of patients with distant hematogenous metastases. Long-term survival after axillary lymph node dissections for metastatic squamous cell carcinoma of the larynx has been reported in 2 previous cases.[7,8] It is currently our practice to recommend a regional lymphadenectomy and local radiotherapy for distant lymphatic metastases when further metastatic workup reveals no other sites of tumor recurrence. If multiple sites of tumor recurrence are documented, then chemotherapy is also considered. However, on our current treatment protocol, only 1 of the 5 patients in this series remains free of disease after regional lymphadenectomy and radiotherapy, with a follow-up of only 1 year.

CONCLUSION

Infraclavicular lymph nodes may become the primary site of lymphatic drainage in patients with head and neck cancer who have undergone previous surgery or radiotherapy to the cervical lymphatics. The metastatic workup for patients with head and neck cancer frequently includes examination of the cervical lymph nodes as well as chest radiography, liver function tests, and a serum calcium level determination. This evaluation may fail to detect metastases to distant lymph nodes in patients who present with recurrent or second primary cancers after previous therapy that has affected the cervical lymphatics. Such patients should undergo careful examination of all major lymph node–bearing regions of the body when being evaluated for the presence of distant metastases.

REFERENCES

1. Vikram B, Srong EW, Shah JP, Spiro R. Failure at distant sites following multimodality treatment for advanced head and neck cancer. Head Neck Surg 1984;6:730-3.
2. Crile GW. Carcinoma of the jaws, tongue, cheek and lips. Surg Gynecol Obstet 1923;36:159-84.
3. Rubenfeld S, Kaplan G, Helder AA. Distant metastases from head and neck cancer. AJR Am J Roentgenol 1962;87:441-8.
4. Gowen GF, Desuto-Nagy G. The incidence and sites of distant metastases in head and neck carcinoma. Surg Gynecol Obstet 1963;116:603-7.
5. Martin HE, Munster H, Sugarbaker ED. Cancer of the tongue. Arch Surg 1940;41:888-936.
6. Troell R, Teris D. Detection of metastases from head and neck cancer. Laryngoscope 1995;105:247-50.
7. Nelson RW, Sisk M. Axillary metastases from carcinoma of the larynx: a 25-year survival. Head Neck 1994;16:83-7.
8. Hoye RC, Herrold K, Smith RR, Thomas LB. A clinicopathological study of epidermoid cancer. Cancer 1962;15:741-9.
9. Merino OR, Lindberg RD, Fletcher GH. Analysis of distant metastasis from squamous cell carcinoma of the upper respiratory and digestive tract. Cancer 1977;40:145-51.
10. Probert JC, Thompson RW. Patterns of spread of distant metastasis in head and neck cancer. Cancer 1974;33:127-33.
11. Leibel DA, Scott CB. The effect of regional control on dissemination in carcinoma of the head and neck. Results of analysis from the head and neck database. Int J Radiat Oncol Biol Phys 1991;21:549-56.
12. Calhoun KH, Fulmer P, Weiss R, Hokanson JA. Distant metastases from head and neck squamous cell carcinoma. Laryngoscope 1994;104:1199-205.
13. Burn JI. Obstructive lymphopathy. In: Gooneratne BW, ed. Lymphography: clinical and experimental. London, England: Butterworths Publishing, 1974:36-7.
14. Rouviere H. Anatomie des lymphatiques de l'homme. Paris, France: Masson, 1932.
15. Bartels P. Das Lymphgefasssystem. In: von Bardeleben K, ed. Handbuch der Anatomie des Menschen. Vol 3. Jena: Gustav Fischer, 1909.
16. Haagensen CD. The head and neck. In: Haagensen CD, ed. The lymphatics in cancer. Philadelphia, Pa: WB Saunders, 1979;59:60-208.
17. Sappey MPC. Anatomie, physiologie, pathologie des vaisseaux lymphatiques considerés chez l'homme et les vertébrés. Paris: A. dela Haye and E. La Crosnier, 1885.

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MONTGOMERY® SALIVARY BYPASS TUBE IN THE RECONSTRUCTION OF THE HYPOPHARYNX

COST-BENEFIT STUDY

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This study analyzes the postoperative complications and the functional results in 61 patients who underwent total laryngectomy with partial or total (circumferential) pharyngectomy reconstructed with a pectoralis major myocutaneous flap, in relation to the use of the Montgomery® Salivary Bypass Tube (MSBPT). There were no significant differences regarding frequency of postoperative cervical complications in relation to the use of the MSBPT. The median hospital stay for patients without the MSBPT (36 days) was significantly higher than that for patients with the MSBPT (25 days). Although the MSBPT did not modify the rate of complications at the cervical level, it did reduce their severity. A financial study showed the cost-effectiveness of using the MSBPT. Systematic use of the MSBPT is recommended after total laryngectomy with partial or total pharyngectomy reconstructed with a pectoralis major myocutaneous flap.

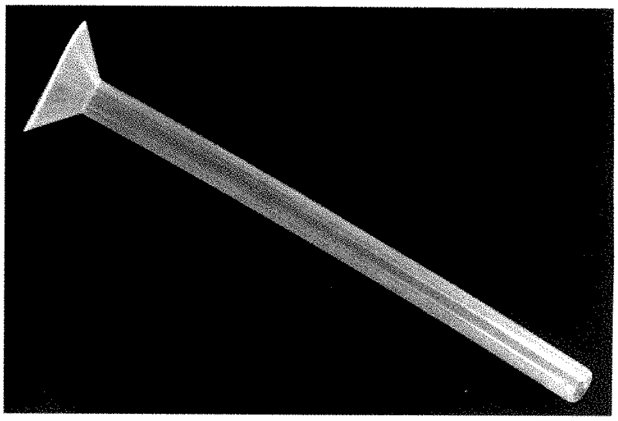
KEY WORDS — complications, hypopharynx carcinoma, Montgomery® Salivary Bypass Tube, pectoralis major myocutaneous flap, pharyngectomy.

INTRODUCTION

The best results in treatment of advanced hypopharyngeal carcinomas are obtained with the combined use of surgery and radiotherapy.[1] Surgery usually implies a total laryngectomy associated with a total (circumferential) or partial pharyngectomy. Following excision of the tumor, the ideal reconstruction method would be that which could be performed in 1 stage, with the best functional results and the lowest mortality and morbidity.[2] The most commonly used method for hypopharyngeal reconstruction at present is free microvascular tissue transplantation, mainly a jejunal loop, but in certain cases the use of myocutaneous flaps is indicated. Following excision of the larynx, hypopharynx, and esophagus, a gastric tube or gastric transposition is used.

Our department uses the pectoralis major myocutaneous flap (PMMF) as the method of choice for reconstructing the hypopharynx following a laryngopharyngectomy. The main advantages of this method are that it is technically simple from a surgical viewpoint and that it provides a highly vital tissue for reconstruction with minimal loss of function. The main inconvenience with this method is the frequency of cervical complications, the most frequent in our experience being pharyngocutaneous fistulas as a result of the difficulty in obtaining adequate healing between the pharynx mucosa and the PMMF. Authors estimate that the frequency of fistula after this kind of reconstruction ranges between 22%[3] and 41%[4] of cases.

In 1978, W. W. Montgomery[5] described the Montgomery® Salivary Bypass Tube (MSBPT; Boston Medical Products, Inc, Westborough, Mass), a silicone tube with an upper, funnel-shaped portion placed in the oropharynx, and an inferior cylindrical portion of various sizes (from 8 to 20 mm diameter) that is passed through the reconstructed hypopharynx to the cervical esophagus[6] (see Figure). The function of the tube consists of directing the saliva toward the distal esophagus, thus preventing saliva from bathing the sutures at the cervical level, and maintaining the calibration of the reconstructed segment.



Montgomery® Salivary Bypass Tube.

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TABLE 1. FREQUENCY AND TYPES OF CERVICAL COMPLICATIONS IN RELATION TO USE OF MSBPT

	No MSBPT (n = 23)	*MSBPT (n = 38)*
No complications	6 (26%)	15 (40%)
Complications	17 (74%)	23 (60%)
Fistula	14	18
No fistula	3	5

MSBPT — Montgomery® Salivary Bypass Tube.

Indications for use of the MSBPT are prevention and treatment of fistulas and strictures produced by surgical and radiotherapeutic treatment of the hypopharynx and cervical esophagus.[7]

The objective of this study was to evaluate the efficiency and the cost-benefit relationship of the MSBPT following total laryngectomy with partial or total pharyngectomy using a PMMF in reconstruction.

PATIENTS AND METHODS

We studied the complications in patients with hypopharyngeal carcinomas subjected to total laryngectomy with partial or total pharyngectomy from 1990 to 1997. The study was retrospective, starting from a database on surgical techniques, postoperative complications, and functional and oncological results obtained in patients with head and neck carcinomas treated in our center.

Forty-six of these patients underwent a total laryngectomy with partial (26 patients) or total pharyngectomy (35 patients). All were men between 39 and 79 years old (average 55.2 years), and all presented locally advanced-stage squamous cell carcinomas (T3 and T4) located at the pyriform sinus (57 patients) or postcricoid area (3 patients). One patient had 2 synchronous tumors: a supraglottic T3N0 tumor and a T1N0 tumor of the posterior hypopharyngeal wall. In 22 patients (36%), surgery was carried out after radiotherapy. Complementary treatment with postoperative radiotherapy was given to all non–previously irradiated patients.

All procedures were carried out by the same surgical team. The technique used was a total laryngectomy with partial or total pharyngectomy associated with radical or functional neck dissection in relation to regional stage. The reconstruction of the posterior hypopharyngeal wall in case of total pharyngectomy was carried out by suturing the esophagus and the oropharynx to the prevertebral fascia, then suturing a split thickness skin graft to the exposed prevertebral fascia portion. The anterior and lateral hypopharyngeal walls were reconstructed by means of a turned-in PMMF, partially tubulated in a horseshoe shape. The lateral skin flap margins were sutured to the cutaneous graft, the superior margin to the base of the tongue, and the inferior margin to the cervical esophagus, according to the technique described by Fabian[2] and Lee and Loré.[8] In the case of partial pharyngectomy, the remaining hypopharyngeal mucosa was sutured to the prevertebral fascia, and a partially tubulated PMMF was placed. The MSBPT was not used in the 22 patients treated between 1990 and 1993, but was placed in all except 1 of the 39 patients treated as of 1993.

In relation to MSBPT use, no significant differences were appreciated between the two groups regarding age, stage, or type of surgery (partial versus total pharyngectomy). Thirty percent of patients in whom MSBPT was not used and 40% in whom the MSBPT was used had received preoperative radiotherapy. There were no significant differences in relation to the use of preoperative radiotherapy between the groups ($p > .05$).

Enteral feeding was maintained at least till the 14th postoperative day, and was then withdrawn if there was no evidence of complications at the cervical level. In cases of fistula or cervical infection, enteral feeding was maintained till resolution of these complications. The MSBPT was maintained following the same criteria as enteral feeding.

We studied the complications that appeared at the cervical level during the postoperative stage, as a function of the use of the MSBPT. When several complications appeared and in order to provide a better understanding of the results, only the major complication was considered.

To analyze the severity of the complications at the cervical level, and following the criteria of Horgan and Dedo,[9] we defined as major fistulas those that persisted longer than 8 weeks and/or required surgical repair. Minor fistulas were considered those that resolved spontaneously within less than 8 weeks. Further, the duration of the hospital stay was considered as an index of the severity of the complications.

Statistical analysis of the quality variables was carried out by means of a χ^2 test or by Fisher's exact test, whereas the quantity variables were evaluated by means of a nonparametric Mann-Whitney U test.

RESULTS

Table 1 shows the frequency and types of complications at the cervical level in relation to use of the MSBPT. Seventy-four percent of patients in whom the MSBPT was not used suffered some kind of complication, the most frequent of which was pharyngocutaneous fistula (61% of patients). In the group in whom the MSBPT was used, 60% of patients pre-

TABLE 2. HOSPITAL STAYS IN RELATION TO USE OF MSBPT AND TO APPEARANCE OF COMPLICATIONS

	No MSBPT (n = 23)			*MSBPT (n = 38)*			
	Median (d)	*Range (d)*	*n*	*Median (d)*	*Range (d)*	*n*	*p*
All patients	36	19-99	23	25	14-58	38	.04
No cervical complications	19.5	18-25	6	16	14-31	15	>.05
Cervical complications	40	20-99	17	28	14-58	23	.008
Fistula	40	20-80	14	28	14-58	18	.03
No fistula	36	29-99	3	25	20-43	5	>.05

sented complications, and the most frequent was again pharyngocutaneous fistula (47% of patients). There were no statistically significant differences in the frequency of complications at the cervical level or of pharyngocutaneous fistula between groups in relation to MSBPT use.

Table 2 shows the median and range of duration of hospital stay in relation to MSBPT use and complications at the cervical level. There was a significant difference in length of hospital stay in relation to the use of the MSBPT (p = .04). This difference is explained by the group of patients that suffered cervical complications: the stay of patients who did not suffer complications showed no significant differences in relation to MSBPT use (p > .05), while the stay for patients who presented cervical complications and in whom the MSBPT was not used was significantly longer than the average stay for patients with cervical complications and the MSBPT (p = .008).

Four patients suffered hemorrhage at the cervical level as an added complication — in all cases associated with a salivary fistula. In 2 patients, the MSBPT was not used. Hemorrhages appeared between postoperative days 5 and 10, corresponding to arterial vessels of the superior and inferior thyroid artery in 2 cases, and to rupture of the internal jugular vein and carotid artery in the other 2. All hemorrhages were solved by surgical revision of the cervical wound. The ligature of the carotid artery in the patient with carotid rupture did not cause neurologic consequences.

Three patients with pharyngocutaneous fistulas required a second surgery to resolve the complication. In all cases, a second PMMF was used. In 2 of these patients, the MSBPT had not been used.

Thirty-six percent (5/14) of pharyngocutaneous fistulas in the group of patients in whom the MSBPT was not used fulfilled criteria of major fistulas, while only 17% (3/18) of fistulas in the group in whom the MSBPT was used fulfilled these criteria. There were no significant differences in the proportion of major fistulas in relation to MSBPT use (p > .05).

Eleven patients (18%) suffered complications at the thoracic level, at the site in which the myocutaneous flap was taken: 5 infections of the thoracic wound (all secondary to pharyngocutaneous fistula or infection in the cervical wound), 4 wound dehiscences, and 2 hemorrhages. One hemorrhage appeared on the second postoperative day in a cirrhotic patient, requiring surgical revision of the thoracic wound. There were no significant differences in the frequency of thoracic complications in relation to MSBPT use (p > .05).

Considering all the patients (n = 61), 72% of patients who had not been irradiated previously and 55% of patients who had received preoperative radiotherapy suffered complications at the cervical level. There were no significant differences in the frequency of complications at the cervical level in relation to previous radiotherapy (p > .05). When we analyzed the frequency of cervical complications in relation to previous radiotherapy in the two groups of patients, with and without MSBPT, there were also no significant differences.

DISCUSSION

According to Fabian,[2] the objectives of reconstruction techniques in oncological head and neck surgery should be 1) not to limit the effectiveness of the ablative technique; 2) to enable reconstruction in a single surgical stage with short hospitalization; and 3) to lower mortality and morbidity rates. In our opinion, an additional objective should be to provide complementary radiotherapy without causing additional problems.

Following total laryngectomy associated with pharyngectomy, there are a variety of reconstruction possibilities that depend on the quantity of resected mucosa. Hui et al[10] established that a pharyngeal remnant of 1.5 cm relaxed or 2.5 cm stretched width was adequate to maintain swallowing function after primary closure. The authors do not specify the use of preoperative or postoperative radiotherapy, which could significantly influence the results obtained. When closure with direct suture is inadvisable, different options may be chosen. According to Loré et al,[11] by using a sliding posterior tongue flap for anterolateral wall repair and a dermal graft for posterior wall repair, it is possible to repair defects of a circular laryngopharyngectomy of up to 8 cm. A higher degree of complexity exists in using myocutaneous flaps, the most used being the PMMF, either with suture to the margins of the residual hypopharyngeal mucosa, in case of partial pharyngectomies, or tubulated, in total pharyngectomies. Fabian[2] recommends partial tubulation in cases of total laryngopharyngectomy, reconstructing the posterior wall by suturing a split thickness skin graft to the prevertebral fascia, and using a partially tubulated PMMF in a horseshoe shape to reconstruct the anterior and lateral walls. Finally, one alternative that is being used with increasing frequency is the microanastomosed jejunal and forearm flap.

We consider that reconstruction with a PMMF is in compliance with the criteria established by Fabian,[2] as it does not limit the surgical exeresis, allows immediate reconstruction, and has a fairly low morbidity in relation to obtaining the flap. Compared to reconstruction with free flaps, it has the advantage of lacking the technical complexity that is inherent to microanastomosis, as well as the possible abdominal morbidity that exists when using a jejunal flap.

Considering that certain reconstruction problems after oncological head and neck surgery are better solved by microanastomosed free flaps, our center is presently developing their application in complex forms of reconstruction.

The main postoperative complication associated with reconstruction with a PMMF is the appearance of fistulas caused by escape of saliva at the suture level of the myocutaneous skin flap in its new cervical location. Such fistulas add a notable morbidity at the cervical wound level.

According to consulted authors, the rate of appearance of fistulas is variable. Lau et al[12] report percentages of fistulization of 8% for total laryngectomy and partial pharyngectomy cases, and 39% for total pharyngolaryngectomy cases reconstructed with a PMMF. Cusumano et al[13] reported a frequency of fistulization of 36%, and Withers et al[3] of 22% for partial and total laryngopharyngectomies together. Fabian,[4] who used a surgical technique similar to ours, including a salivary tube, described a fistulization frequency of 41%. This fistulization problem at the interface between mucosa and skin is not exclusive to the hypopharynx. In a review by Shah et al[14] on the complications associated with the use of 214 PMMFs, with preferential use at the oral cavity and oropharynx, the global rate of fistulization was 29%.

At medium term, one of the complications that might appear after reconstructing the hypopharynx with a PMMF is dysphagia associated with hypopharyngeal stenosis, usually at the level of the inferior suture of the PMMF to the esophagus mucosa. In order to prevent this complication, Lam et al[15] point out that this inferior suture, rather than being circular, should be made with interdigitations that would limit the tendency to circular contracture.

The MSBPT is designed to solve these problems: on one hand, the saliva does not bathe the suture as it circulates through the tube, and on the other hand, the MSBPT maintains the calibration of the suture of the flap with the esophagus. In case of fistulization, as most saliva would circulate through the MSBPT, the flow from the fistula is expected to be less, thus favoring spontaneous resolution.

The rate of fistulization in our study is higher than results in the literature, corresponding to 61% of patients in whom MSBPT was not used, and 47% when it was used. We think that this higher frequency of complications may be due to the general poor state of our patients with hypopharyngeal carcinomas: almost all of them were frequent tobacco and alcohol users, had poor nutrition, and presented advanced tumors requiring extended exeresis, in many cases following recurrence at the local level after radiotherapy.

There is accord between different authors that the appearance of postoperative complications, especially pharyngocutaneous fistulas, prolongs the hospital stay.[16-19] The duration of the hospital stay and the need for new surgical procedures are parameters that we can use to assess the severity of complications associated with head and neck surgery.

The use of the MSBPT did not cause a significant decrease in the frequency of cervical complications or fistulization, but a tendency toward less severity was appreciated: 39% of the fistulas in the group in which the MSBPT was not used met the criteria of major fistula, as compared to 17% in the group in which the MSBPT was used.

Taking the length of hospital stay as a parameter to measure morbidity associated with complications, some significant differences were observed in relation to the use of the MSBPT: the median length of stay when the MSBPT was not used was 36 days, whereas it was 25 days when it was used ($p = .04$). The significant differences in the periods of hospital stay were mainly due to the group of patients presenting fistulas. Considering only those patients whose cervical complication was a fistula, the median length of hospital stay when the MSBPT was not used was 40 days, and 28 days when it was used (p = .008). Neither the group with no complications nor those patients with complications other than fistula showed significant differences in the average length of hospital stay in relation to the MSBPT.

In those patients who presented a fistula and in whom the MSBPT was used, an additional advantage was that with the reduction of salivary flow through the fistula, there was a clear decrease in the need for daily wound care and dressings, which contributed to greater patient comfort.

We agree with Keidan and Kusiak[20] that the use of a PMMF on previously irradiated ground does not increase the frequency of complications at the cervical level.

The price of an MSBPT for our center is equivalent to 1.9 days' hospital stay. Considering that the difference between median hospital stays between the groups of patients with respect to use of the MSBPT was 11 days, the use of the MSBPT in this kind of surgery proves to be cost-effective.

The only inconvenience with the MSBPT as reported by patients was the sensation of a foreign body in the oropharynx on swallowing, causing discomfort of various degrees.

In spite of the fact that our study includes a limited number of patients and that we did not assign the use of the MSBPT in a randomized way, given the uniformity of the disease involved and since all the patients were consecutively operated on by the same surgical team using the same surgical technique, we consider that conclusions can be drawn. The use of the MSBPT in reconstruction of the hypopharynx with a PMMF following total laryngectomy with partial or total pharyngectomy, though not contributing to significantly reduce the rate of postoperative cervical complications, does decrease their severity and the period of hospital stay. The cost-benefit ratio clearly favors the use of the MSBPT. We therefore recommend its systematic use in this kind of intervention.

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REFERENCES

1. Quer M, Esteller E, León X, et al. Tratamiento del carcinoma del seno piriforme en el hospital de la Santa Creu i Sant Pau de Barcelona: papel de la cirugía. An Otorrinolaringol Ibero Am 1993;20:459-78.

2. Fabian RL. Reconstruction of the laryngopharynx and cervical esophagus. Laryngoscope 1984;94:1334-50.

3. Withers EH, Franklin JD, Madden JJ, Lynch JB. Immediate reconstruction of the pharynx and cervical esophagus with the pectoralis major myocutaneous flap following laryngopharyngectomy. Plast Reconstr Surg 1981;68:898-904.

4. Fabian RL. Pectoralis major myocutaneous flap reconstruction of the laryngopharynx and cervical esophagus. Laryngoscope 1988;98:1227-31.

5. Montgomery WW. Salivary bypass tube. Ann Otol Rhinol Laryngol 1978;87:159-62.

6. Montgomery WW. Current modifications of the salivary bypass tube and tracheal T-tube. Ann Otol Rhinol Laryngol 1986;95:121-5.

7. Montgomery WW, Montgomery SK. Manual for use of Montgomery® laryngeal, tracheal, and esophageal prostheses: update 1990. Ann Otol Rhinol Laryngol Suppl 1990;99(suppl 150):19-22.

8. Lee KY, Loré JM. Two modifications of pectoral major myocutaneous flap (PMMF). Laryngoscope 1986;96:363-7.

9. Horgan EC, Dedo HH. Prevention of major and minor fistulae after laryngectomy. Laryngoscope 1979;89:250-60.

10. Hui Y, Wei WI, Yuen PW, Lam LK, Ho WK. Primary closure of pharyngeal remnant after total laryngectomy and partial pharyngectomy: how much residual mucosa is sufficient? Laryngoscope 1996;106:490-4.

11. Loré JM Jr, Klotch DW, Lee KY. One-stage reconstruction of the hypopharynx using myomucosal tongue flap and dermal graft. Am J Surg 1982;144:473-6.

12. Lau WF, Lam KH, Wei WI. Reconstruction of hypopharyngeal defects in cancer surgery: do we have a choice? Am J Surg 1987;154:374-80.

13. Cusumano RJ, Silver CE, Strauch B. Pectoralis myocutaneous flap for replacement of cervical esophagus. Head Neck 1989;11:450-6.

14. Shah JP, Haribhakti V, Loree TR, Sutaria P. Complications of the pectoralis major myocutaneous flap in head and neck reconstruction. Am J Surg 1990;160:352-5.

15. Lam KH, Wei WI, Lau WF. Avoiding stenosis in the tubed greater pectoral flap in pharyngeal repair. Arch Otolaryngol Head Neck Surg 1987;113:428-31.

16. McCombe WM, Jones AS. Radiotherapy and complications of laryngectomy. J Laryngol Otol 1993;107:130-2.

17. Arriaga MA, Kanel KT, Johnson JT, Myers EN. Medical complications in total laryngectomy: incidence and risk factors. Ann Otol Rhinol Laryngol 1990;99:611-5.

18. Kraus DH, Pfister DG, Harrison LB, et al. Salvage laryngectomy for unsuccessful larynx preservation therapy. Ann Otol Rhinol Laryngol 1995;104:936-41.

19. Huang RD, Silver SM, Hussain A, Parnes S, Wing PD. Pectoralis major myocutaneous flap: analysis of complications in a VA population. Head Neck 1992;14:102-6.

20. Keidan RD, Kusiak JF. Complications following reconstruction with the pectoralis major myocutaneous flap: the effect of prior radiation therapy. Laryngoscope 1992;102:521-4.

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HISTOCHEMICAL AND IMMUNOCYTOCHEMICAL STUDY OF NITRERGIC INNERVATION IN HUMAN NASAL MUCOSA

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Nitric oxide (NO) is a free radical gas that has been found to be produced in neuronal cells by the action of the enzyme brain nitric oxide synthase (bNOS). The aim of this study was to identify NO-containing nerve structures in the human nasal mucosa by localizing bNOS and to find out whether NO production is attached to the parasympathetic system. For this purpose, immunocytochemistry with antibodies to bNOS and neurofilament was performed. Additionally, nicotinamide-adenine dinucleotide phosphate diaphorase (NADPH-d), an enzyme that correlates with the localization of NO synthase, and acetylcholinesterase were visualized in a histochemical double staining technique on frozen sections. The NADPH-d and bNOS reactions were found in axons of nerve bundles and in subepithelial, glandular, and vascular nerve fibers. Arteries showed a distinctly developed nitric innervation, whereas no activity was found in nerve fibers supplying veins. A high coexistence of NADPH-d in parasympathetic nerves could be detected. These findings suggest that NO takes part in the nerve control functions of the human nasal mucosa.

KEY WORDS — brain nitric oxide synthase, human nasal respiratory mucosa, nicotinamide-adenine dinucleotide phosphate diaphorase, nitrergic innervation, nitric oxide.

INTRODUCTION

The different functions of the nasal mucosa are regulated by a variety of endogenous and exogenous factors. In addition to classic and peptidergic neurotransmitters, another transmitter is found increasingly to be important in the neuronal control of the various physiological processes.[1-3] It is well known that nitric oxide (NO) acts as a neuronal transmitter in the central and peripheral nervous system.[4] Studies performed on rat nasal tissue samples demonstrated endogenous NO production in nerve fibers by enzyme labeling techniques.[5,6] Further, it was assumed that NO plays a certain role in olfaction.[7-9] Additionally, this endogenously formed free radical gas is supposed to have a variety of other important effects, eg, on blood flow regulation and human immunology. Nitric oxide influences macrophage activity and has certain antiviral properties.[10] The physiological concentration of airway-derived NO in healthy subjects is high enough to have bacteriostatic effects. It is one of the most important factors in maintaining a low bacterial level in the paranasal sinuses of humans.[11] There is some evidence that NO increases the ciliary beat frequency of the respiratory epithelium in defined concentrations.[12] Additionally, Puybasset et al[13] have been able to demonstrate that the pulmonary blood flow is influenced by NO.

Nitric oxide is produced by nitric oxide synthase (NOS) using L-arginine as a substrate. Three different isoforms of the enzyme have been cloned so far.[14] Neuronal or brain NOS (bNOS) and epithelial NOS (eNOS) are dependent on calcium influx; the third isoform (independent NOS; iNOS), however, is independent of elevated calcium levels and can be induced by cytokines and bacterial lipopolysaccharide.

Nitric oxide synthases have been correlated with a reactivity for nicotinamide-adenine dinucleotide phosphate diaphorase (NADPH-d).[15,16] Comparing the staining patterns of both enzymes in different mammals demonstrated that even though all NOSs possess NADPH-d activity, not all NADPH-d activity is due to NOS activity.[4,17,18]

Nitric oxide is chemically instable and therefore difficult to detect. Thus, its importance as a neurotransmitter could only be noticed after purification of the enzyme bNOS, from which specific antisera could be obtained.[19] Already in 1987, Palmer et al[20] had found evidence for NO's being identical to a substance known at that time as endothelium-derived relaxing factor. This molecule was known to diffuse from the endothelium to mediate the relaxation of

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blood vessels. Although the histochemical procedure showing the activity of NADPH-d with nitroblue tetrazolium and nicotinamide-adenine dinucleotide phosphate (NADPH) had been developed earlier, coexistence of this factor and NOS in many tissues was noticed only a few years ago, when NOS antisera became available.[18,21,22] Since then, the location of NADPH-d activity and the immunoreactivity of the different NOS isoforms have been investigated in various tissues, indicating a role of NO in, for example, smooth muscle relaxation.

The target of this study was to identify NO-producing nerve structures in the human nasal mucosa by localizing NADPH-d with the histochemical procedure first described by Vincent and Kimura[15] and bNOS immunocytochemistry. Since bNOS or NADPH-d is frequently found in parasympathetic neurons, a double-staining procedure was performed to detect a possible correlation of NO and cholinergic axons in human nasal mucosa.[23,24]

MATERIAL AND METHODS

Sampling of Nasal Mucosa. Specimens of inferior turbinates were received from 42 patients (32 men, 10 women; 22 to 59 years; mean age 35.3) who required nasal surgery due to mechanically induced nasal congestion (hypertrophic concha or septal deviation). Tissue samples for histochemistry were fixed in 4% phosphate-buffered paraformaldehyde solution for 2 hours at 4°C and cryoprotected in sucrose solution, snap-frozen in liquid nitrogen, and stored at –20°C. Finally, sections (8 to 15 µm thickness) were cut with a cryostat (Reichart-Jung). For bNOS immunocytochemistry, unfixed samples were snap-frozen in liquid nitrogen and sections of 5 to 9 µm were cut. For morphological and clinical evaluation, the first sections were stained with Mayer's hemalum. Inflamed tissue was excluded.

Immunocytochemistry. Cryosections were air-dried for 30 minutes, fixed in ice-cold acetone for 10 minutes, and air-dried for 30 minutes. After blocking of endogenous peroxidase by immersion in 0.002% hydrogen peroxide solution in phosphate-buffered saline (PBS), the sections were preincubated with normal horse serum (neurofilament) or normal goat serum (bNOS) in PBS. For detection of bNOS, sections were incubated at room temperature with an antibody raised against neuronal NOS isolated from rat cerebellum diluted 1:100 at room temperature for 1 hour or 1:1,500 overnight (4°C). Alternatively, an antibody raised against a protein fragment of human bNOS (Transduction Laboratories, Lexington, Ky) diluted 1:100 overnight (4°C) was used. Serial sections were incubated at room temperature for 1 hour with a monoclonal antibody to neurofilament (dilution 1:100) reacting with the phosphorylated form of the 200 kd and the 70 kd components of the 3 major polypeptide subunits (DAKO Diagnostika, Hamburg, Germany). Controls consisted of omitting the primary antibody in the staining procedure and incubating serial sections with rabbit IgG (DAKO Diagnostika) diluted to the same concentration as the NOS antibody. No specific immunoreactions were observed. Biotinylated anti-rabbit IgG (bNOS) or anti-mouse IgG (neurofilament) at a dilution of 1:200 was applied for 30 minutes at room temperature. Finally, the immunocomplexes were visualized by the avidin-biotin-complex method and the chromogen aminoethylcarbazol. At the end of the immunocytochemical procedures, counterstaining with Mayer's hemalum was performed.

Histochemistry. Reactivity to NADPH-d was demonstrated by the histochemical method of Vincent and Kimura,[15] modified by Neuhuber et al.[25] Frozen sections were immersed in a solution consisting of nitroblue tetrazolium (0.1 mg/mL), β-NADPH (1.0 mg/mL), and Triton-X 100 (0.3%) in phosphate buffer for 50 minutes. Control sections were prepared by omitting β-NADPH. Following NADPH-d histochemistry, cholinergic structures were localized with the method for acetylcholinesterase (AChE) first described by Karnovsky and Roots.[26] For this double-staining procedure, sections were incubated with sodium citrate (0.1 mol), cupric sulfate (0.03 mol), potassium-ferricyanide (0.005 mol), tetraisopropyl pyrophosphoramide, and acetylthiocholine iodide dissolved in sodium hydrogen-maleate buffer (0.1 mol, pH 6.0) for 40 minutes. Finally, all sections were mounted with Kaiser's glycerin gelatin, evaluated, and documented through bright-field microscopy.

RESULTS

The NADPH-d histochemistry showed positive reactions in epithelial, endothelial, and neuronal structures. Enzyme activity was located in the cytoplasm of mucosal, but also of ductal and glandular, epithelial cells. Compared to the epithelium, the endothelium of arteries and veins showed a weaker staining intensity. Positive nerve fibers were distributed in all regions of the human nasal inferior turbinate mucosa. They could be detected in thick nerve bundles adjacent to the turbinate bone (Fig 1A) as well as in smaller axon bundles. Especially in dense nerve plexuses around thick arteries, reactivity was observed (Fig 2). However, it was impossible to demonstrate NADPH-d in the few nerve fibers supplying the venous vessels of the nasal cavernous tissue. The neuronal network of the acini of seromucous glands showed NADPH-d reactivity (Fig 3A). Additionally,

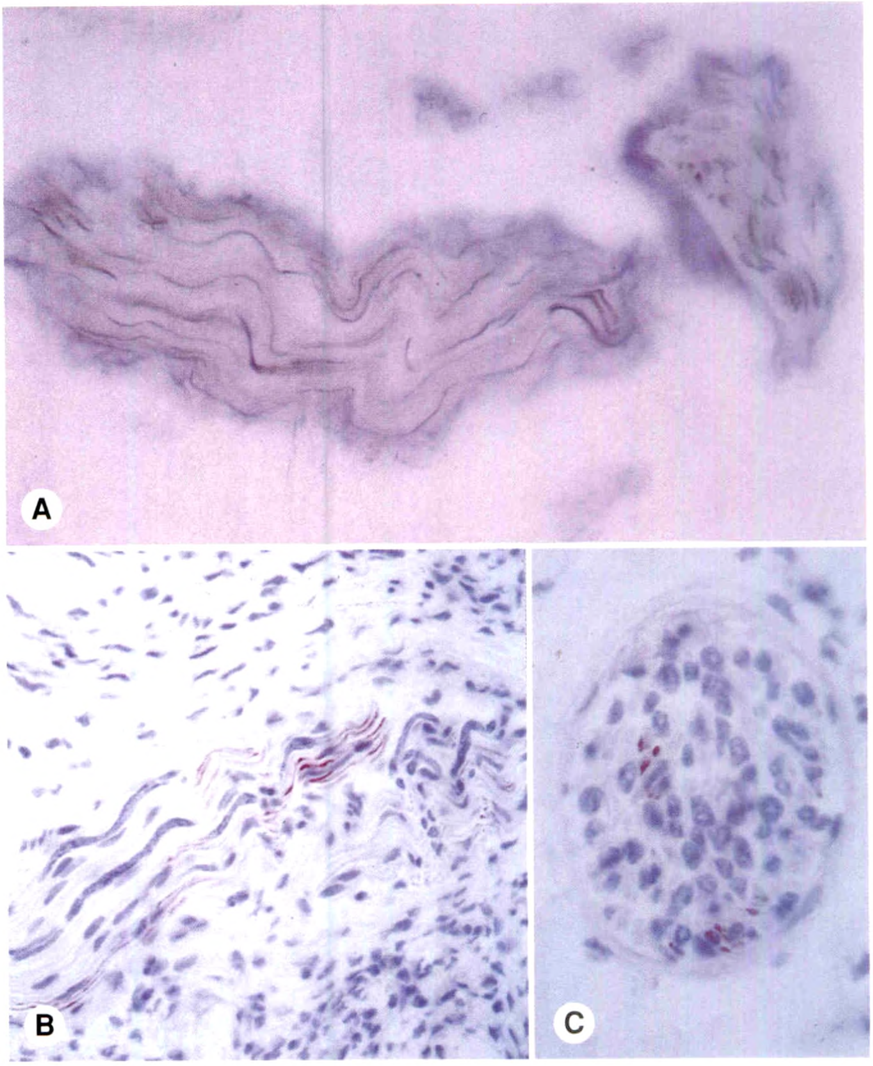

Fig 1. Thick nerve bundles in nasal mucosa of humans. A) Acetylcholinesterase (AChE) and nicotinamide-adenine dinucleotide phosphate diaphorase (NADPH-d) histochemistry (original ×320). Shows evident colocalization of both enzymes (AChE, brown; NADPH-d, blue). B) Immunocytochemistry of brain nitric oxide synthase (bNOS)–positive (red) longitudinally cut axons (avidin-biotin-complex [ABC] technique, Mayer's hemalum, original ×200). C) Cross section of nerve with some bNOS-immunoreactive axons (ABC technique, Mayer's hemalum, original ×400).

fine nerve fibers around excretory ducts could be stained (Fig 3B). The NADPH-d–positive fibers in the subepithelial layer were found very close to the epithelium in some cases; however, they never penetrated into the epithelium (Fig 4). The double staining procedure (NADPH-d–AChE) revealed a coexistence

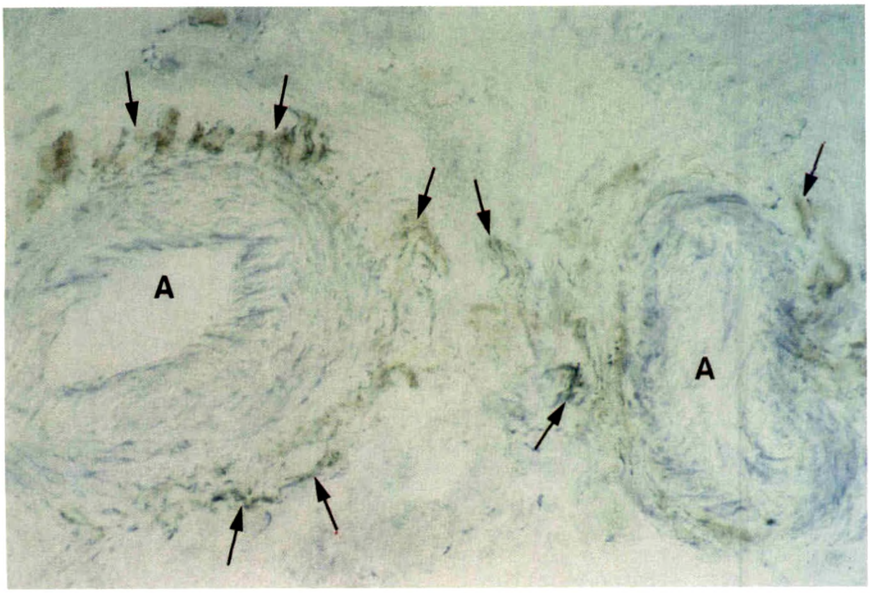

Fig 2. Transversely cut arterial vessels (A; original ×160). AChE–NADPH-d double staining of periarterial nerve fibers. More cholinergic (brown) than nitrergic (blue) axons (arrows) are present.

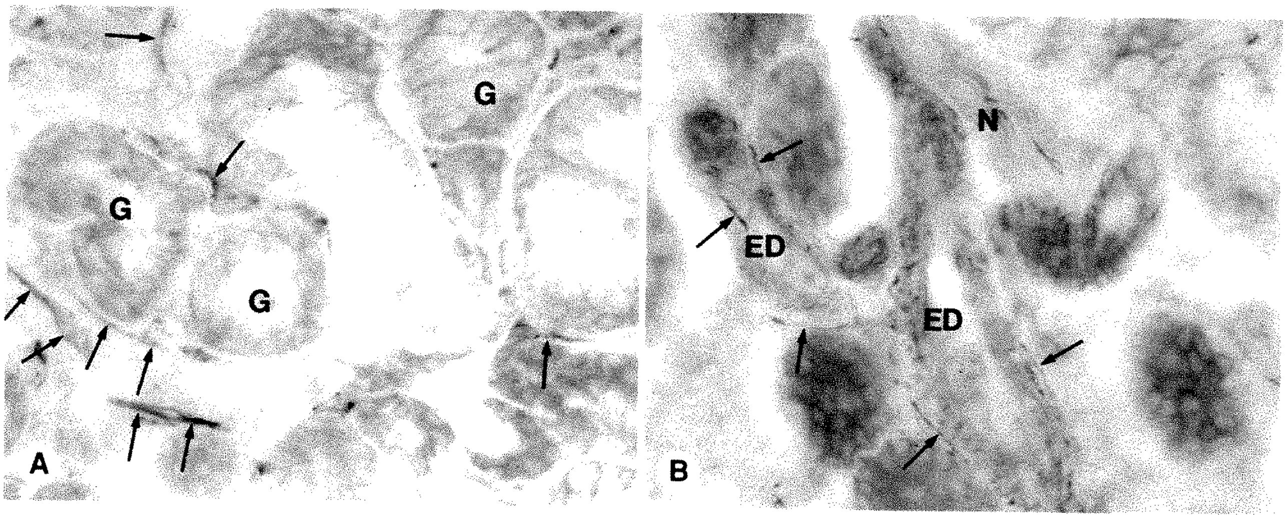

Fig 3. Nitrergic innervation in human nasal glandular structures (original ×160). A) Fine network of AChE- and NADPH-d–stained fibers (arrows) around acini of seromucous glands (G). B) Single NADPH-d–positive nerve fibers (arrows) running along excretory ducts (ED) of nasal gland. Shows adjacent nerve bundle (N) with some positive axons.

of both enzyme activities in axons of the lamina propria of the respiratory nasal mucosa. As expected, AChE was also located in neurons that did not show NADPH-d staining (Figs 1-4). Brain NOS immunocytochemistry confirmed the results found in histochemistry. With this method, NO neurotransmitter synthesis could be demonstrated in thick nerve bundles (Figs 1B and 5A) as well as in subepithelial and arterial nerve fibers (Figs 2 and 6A). In addition, immunocytochemistry with antibodies to neurofilament on serial sections was used to confirm the neuronal localization of bNOS immunoreactivity (Figs 5B and 6B). This technique was useful in facilitating anatomic orientation and in estimating the number of axons labeled by neuronal NOS antibodies. By summarizing our histochemical and immunocytochemical observations on enzymes of NO synthesis in nerves, we can assume that small amounts of the neurotransmitter NO are distributed in all regions of the nasal mucosa.

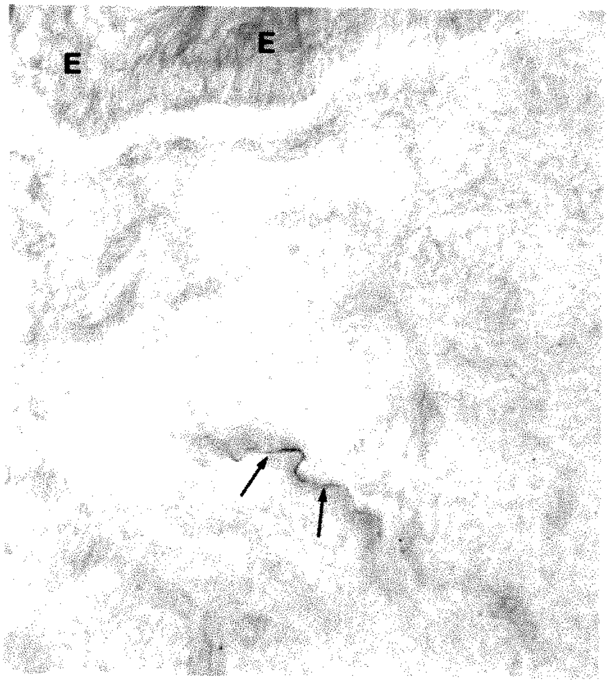

Fig 4. AChE–NADPH-d double staining of solitary subepithelial nerve fiber (arrows; original ×250). E — epithelium.

DISCUSSION

Since NADPH-d is generally considered an indirect marker for all NOS isoforms, histochemical labeling of this enzyme provides an overview of all NO-producing structures, including neuronal, endothelial, and epithelial cells.[15,16] Furthermore, histochemical NO tracing could be confirmed by bNOS immunocytochemistry. To specify neuronal NO synthesis, it was necessary to compare the bNOS findings to serial sections stained by neurofilament immunocytochemistry. Thus, it was possible to visualize NO in axons of human inferior turbinate with histochemical and immunocytochemical techniques. Additionally, the results of double staining histochemistry (NADPH-d–AChE) were consistent with findings made in other organs, showing that NO is localized in parasympathetic structures.

Neuronal NO Production. In a variety of tissues, neuronal NO production has already been histochemically and/or immunohistochemically demonstrated. Positive nerve fibers have been detected, eg, in the blood vessels of the brain,[27] the hypothalamic portal system,[28] and the tongue.[29] In the respiratory tract, nitrergic nerve fibers have been found in the trachea and lung of guinea pigs.[29,30]

Kulkarni et al[6] investigated nitrergic innervation in nasal mucosa of rats and humans by using histo-

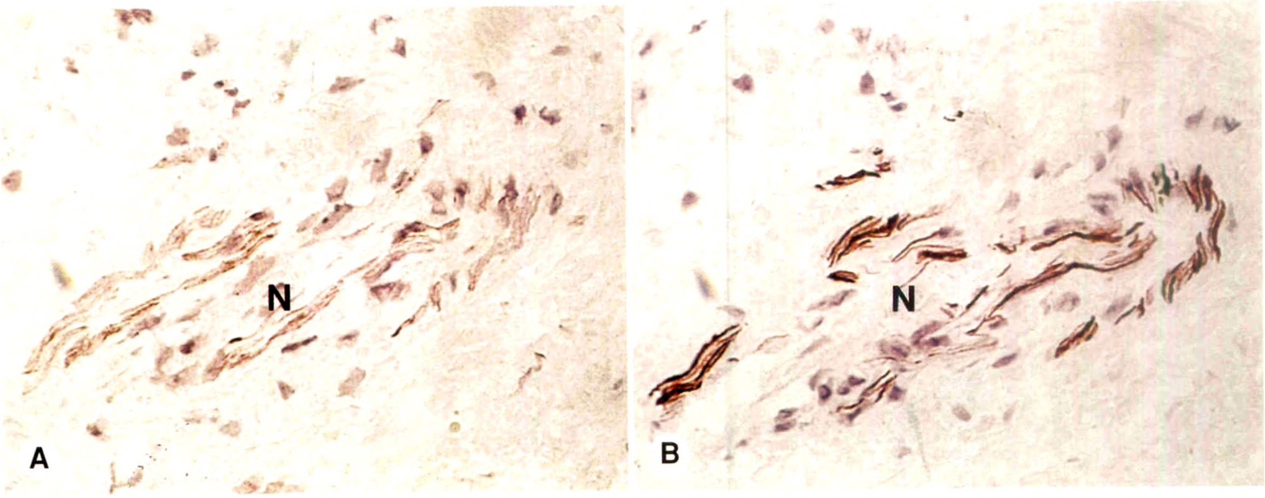

Fig 5. Longitudinally cut smaller nerve bundles (N; ABC technique, Mayer's hemalum, original ×200). A) Immunocytochemical reaction (brown) to bNOS. B) Serial section of same nerve bundles marked by neurofilament antibodies.

chemical and immunocytochemical techniques. They reported that NOS immunoreactivity is mainly localized in perivascular nerve fibers.[6] In our samples, we found NOS-reactive nerve bundles near the seromucous glands and some single axons around acini, as already observed on histochemistry. Fibers running parallel to the epithelium were observed in most of the cases. Perivascular nerve fibers predominated

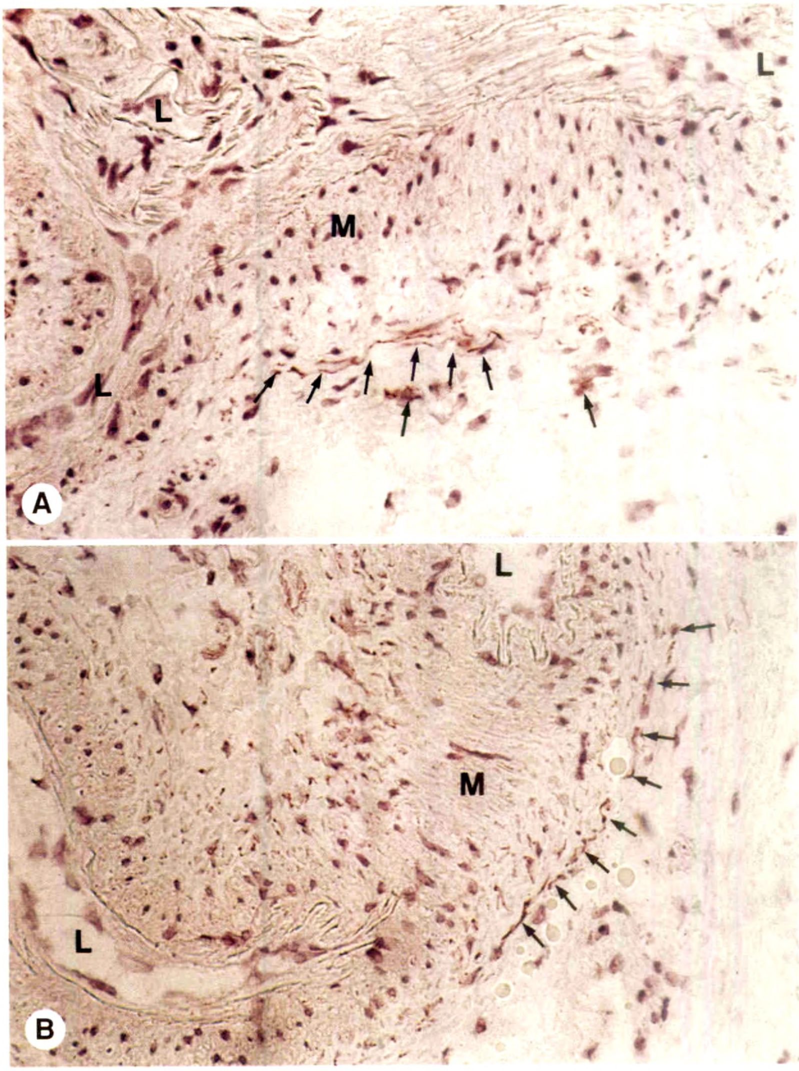

Fig 6. Nitrergic innervation pattern (arrows) of 2 (A,B) longitudinally cut arteries (ABC technique, Mayer's hemalum, original ×160). L — lumen; M — tunica media.

in the adventitia of arteries of various calibers, but were not found in venous vessels. This vascular innervation, however, was distributed in the entire lamina propria of the human nasal mucosa and not only in the deep vascular layer, as described by Kulkarni et al.[6] The present results can possibly be explained by the various localizations and sampling procedures of the tissue.[17] Kulkarni et al[6] used postmortem material after different periods of time, they did not specify the site of the nasal mucosa the samples were taken from, the cryosections were thicker (15 μm), and the fixation solution was different. Thus, our findings suggest more extensive innervation in humans.

In rats, however, Kulkarni et al[6] and Hanazawa et al[5] found a more dense nitrergic innervation in blood vessels and glands. Hanazawa et al[5] described NADPH-d–reactive nerve fibers distributed all over the lamina propria. Dense nerve fiber plexuses were detected around the acini of seromucous glands and arterial blood vessels. In contrast to our findings in humans, they even described positive nerve fibers surrounding smaller blood vessels such as venules and capillaries.[5] Hanazawa et al[5] and Kulkarni et al[6] also found nerve fibers in the respiratory epithelium of the nasal mucosa of rats, which could not be detected in our sections of human tissue.

Nitrergic-Cholinergic Coexistence. Our studies showed a striking colocalization of NADPH-d and AChE in all nerve structures of the nasal mucosa by applying a double staining procedure. Similarly, in investigating the nitrergic innervation of the pancreas in rats and humans, it was also presumed that the labeled fibers were of parasympathetic origin.[17] In the esophagus of humans and other species, a nitrergic-cholinergic coexistence of enteric neurons could be found by NADPH-d–AChE double staining and NOS immunocytochemistry.[24,25]

Working on rat tissue samples, Hanazawa et al[5] investigated the expression of NADPH-d in the nasal mucosa and its ganglia in the rat. They demonstrated NADPH-d activity in all perikarya of the pterygopalatine ganglion. This ganglion contains the neurons supplying the parasympathetic petrosus minor nerve, which innervates the nasal mucosa. In contrast to these findings, there were no reactions in the nerve cell bodies of the sympathetic superior cervical ganglion. Some neurons in the gasserian sensory ganglion, however, could be labeled. Considering the distribution of neuronal structures in the ganglia leading to the nasal cavity, it seems likely that NO participates in neurotransmission of the parasympathetic nervous system and parts of the sensory nervous system of rat nasal mucosa.[5] The colocalization of AChE and NADPH-d in humans as observed in our study underlines that the messenger molecule NO should be assigned to the parasympathetic nervous system.

Nitric Oxide as Sensory Transmitter. Nitric oxide is also said to act as a transmitter molecule in sensory neurons. Aimi et al[31] described NADPH-d–positive neurons in the sensory ganglia of the rat. Also, in the lower airways of the guinea pig, the existence of nitrergic sensory neurons was discussed.[30] It has been supposed that NADPH-d–labeled neurons in the gasserian ganglion are identical to the perikarya of sensory nerve fibers from nasal mucosa.[5] Although it is still controversial whether NOS is located in olfactory receptor neurons, it has been suggested that NO at least modulates olfactory sensory transduction through perireceptor events.[6-9]

Within our study we could not find any differences between the location of bNOS in immunocytochemistry and the nerve structures stained with NADPH-d histochemistry. Nevertheless, there have been differences described by several authors, who did not find a strict correlation between NADPH-d enzyme activity and neuronal NOS immunoreactivities.[6,17,18] A likely explanation for the inconstant correlation might be that NADPH-d can possibly be expressed without being dependent on NOSs,[32] and that some other enzymes such as oxygenases and nonspecific phosphatases can catabolize NADPH.[33] It cannot be excluded, either, that NADPH-d may be associated with some still-unknown NOS isoenzyme that does not react to any of the antisera used.

Summarizing, we can say that by histochemical and immunocytochemical techniques, enzymes important for NO synthesis (NADPH-d; bNOS) could be detected in nerves of the human nasal mucosa. Additionally, a histochemical double staining procedure showed a close relationship between NADPH-d–positive nerve fibers and the cholinergic nerves. Compared to other histochemical (AChE) and immunocytochemical markers (eg, tyrosine hydroxylase), the staining intensity of neuronal NO enzymes seemed to be less intense; nevertheless, they could be observed in axons widely distributed in nasal mucosa. These findings lead us to propose that NO also acts as a neurotransmitter in the human nasal mucosa and can be assigned to parasympathetic nerve structures.

REFERENCES

1. Riederer A, Fischer A, Knipping S, Unger J, Lange W, Kastenbauer E. Basic innervation pattern and distribution of classic autonomic neurotransmitters in human nasal vasculature. Laryngoscope 1996;106:286-91.

2. Baraniuk JN, Lundgren JD, Okayama M, et al. Vasoactive intestinal peptide in human nasal mucosa. J Clin Invest 1990; 86:825-31.

3. Uddman R, Malm L, Sundler F. Peptide containing nerves in the nasal mucosa. Rhinology 1981;19:75-9.

4. Bredt DS, Snyder SH. Nitric oxide, a novel neuronal messenger. Neuron 1992;8:3-11.

5. Hanazawa T, Motosugi H, Konno A, Kaneko T, Tanaka K, Chiba T. Distribution of NADPH-diaphorase positive nerve fibers in the rat nasal mucosa. Neurosci Lett 1993;159:71-4.

6. Kulkarni AP, Getchell TV, Getchell ML. Neuronal nitric oxide synthase is localized in extrinsic nerves regulating perireceptor processes in the chemosensory nasal mucosa of rats and humans. J Comp Neurol 1994;345:125-38.

7. Breer H, Klemm T, Boekhoff I. Nitric oxide mediated formation of cyclic GMP in the olfactory system. Neuroreport 1992;3:1030-2.

8. Breer H, Shepherd GM. Implications of the NO/cGMP system for olfaction. Trends Neurosci 1993;16:5-9.

9. Lischka FW, Schild D. Effects of nitric oxide upon olfactory receptor neurons in *Xenopus laevis*. Neuroreport 1993; 4:582-4.

10. Nathan C. Nitric oxide as a sensory product of mammalian cells. FASEB J 1992;6:3051-64.

11. Lundberg JON, Farkas-Szallasi T, Weitzberg E, Rinder J, Lidholm J, Änggard A. High nitric oxide production in human paranasal sinuses. Nat Med 1995;1:370-3.

12. Jain B, Rubinstein I, Robbins RA, Leise KL, Sisson JH. Modulation of airway epithelial cell beat frequency by nitric oxide. Biochem Biophys Res Commun 1993;91:83-8.

13. Puybasset L, Rouby JJ, Mourgeon E, et al. Inhaled NO in ARDS: dose response curves. Br J Anaesthesiol Suppl 1994; 72(suppl 1):56-60.

14. Geller DA, Lowenstein CJ, Shapiro RA, et al. Molecular cloning and expression of inducible nitric oxide synthase from hepatocytes. Proc Natl Acad Sci U S A 1993;90:3491-5.

15. Vincent SR, Kimura H. Histochemical mapping of nitric oxide synthase in the rat brain. Neuroscience 1992;46:748-55.

16. Forster ER, Southham E. The intrinsic and vagal extrinsic innervation of the rat stomach contains nitric oxide synthase. Neuroreport 1993;4:275-8.

17. Wörl J, Wiesand M, Mayer B, Greskötter K-R, Neuhuber WL. Neuronal and endothelial nitric oxide synthase immunoreactivity and NADPH-diaphorase staining in rat and human pancreas: influence of fixation. Histochemistry 1994;102:353-64.

18. Dawson TM, Bredt DS, Fothui M, Hwang PM, Snyder SH. Nitric oxide synthase and neuronal NADPH diaphorase are identical in brain and peripheral tissues. Proc Natl Acad Sci U S A 1991;88:7797-801.

19. Bredt DS, Snyder SH. Isolation of nitric oxide synthase, a calmodulin requiring enzyme. Proc Natl Acad Sci U S A 1990; 87:682-5.

20. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987;327:524-6.

21. Thomas C, Pearse AGE. The solitary active cells. Histochemical demonstration of damage resistant nerve cells with a TPN-diaphorase reaction. Acta Neuropathol (Berl) 1964;3:238-49.

22. Bredt DS, Glatt CE, Hwang PM, Fothui M, Dawson TM, Snyder SH. Nitric oxide synthase protein and mRNA are discretely localized in neuronal populations of the mammalian CNS together with NADPH diaphorase. Neuron 1991;7:615-24.

23. Grozdanovic Z, Baumgarten HG, Brüning G. Histochemistry of NADPH-diaphorase, a marker for neuronal nitric oxide synthase, in the peripheral autonomic nervous system of the mouse. Neuroscience 1992;48:225-35.

24. Sörensen B, Neuhuber WL, Mayer B, Wörl J. Nitrerge Co-Innervation motorischer Endplatten im Ösophagus bei Mensch und Schwein. Ann Anat Suppl 1995;177(suppl):214.

25. Neuhuber WL, Wörl J, Berthoud HR, Conte B. NADPH-diaphorase–positive nerve fibres associated with motor endplates in the rat esophagus: new evidence for co-innervation of striated muscle by enteric neurons. Cell Tissue Res 1994;276: 23-30.

26. Karnovsky MJ, Roots L. A "direct coloring" thiocholine method for cholinesterases. J Histochem Cytochem 1964;12: 212-21.

27. Bredt DS, Hwang PM, Snyder S. Localization of nitric oxide synthase indicating a neuronal role for nitric oxide. Nature 1990;347:768-70.

28. Ceccatelli S, Lundberg JM, Zhang X, Aman K, Hökfelt T. Immunohistochemical demonstration of nitric oxide synthase in the peripheral autonomic nervous system. Brain Res 1994; 656:381-95.

29. Kummer W, Fischer A, Mundel P, et al. Nitric oxide synthase in VIP-containing vasodilatator nerve fibres in the guinea-pig. Neuroreport 1992;3:653-5.

30. Fischer A, Mundel P, Mayer B, Preissler U, Philippin B, Kummer W. Nitric oxide synthase in guinea pig lower airway innervation. Neurosci Lett 1993;149:157-60.

31. Aimi Y, Fujimura M, Vincent SR, Kimura H. Localization of NADPH-diaphorase–containing neurons in sensory ganglia of the rat. J Comp Neurol 1991;306:382-92.

32. Matsumoto T, Nakane M, Pollock JS, Kuk JE, Förstermann U. A correlation between soluble brain nitric oxide synthase and NADPH-diaphorase activity is only seen after exposure of the tissue fixative. Neurosci Lett 1993;155:61-4.

33. Gossrau R. Further studies on the usefulness of the NADPH-tetrazolium salt system for the visualization of nitric oxide synthase (NOS). Ann Anat Suppl 1994;176(suppl):91.

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UNILATERAL TONSILLAR ENLARGEMENT AND TONSILLAR LYMPHOMA IN CHILDREN

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The clinical presentation and surgical and pathological findings of 46 children with unilateral tonsillar enlargement (UTE; age range 2 to 13 years, mean age 6.5) who underwent tonsillectomy for biopsy purposes between 1975 and 1995 were compared with those of 7 children who received treatment for tonsillar lymphoma (TL; age range 2 to 9 years, mean age 4.8) during the same period. There was no history of rapid tonsillar enlargement in children in the UTE group, and only 20 (43%) were symptomatic. Symptoms included recurrent sore throats in 10 patients (22%), snoring in 5 (11%), nasal obstruction in 4 (9%), and dysphagia in 1 (2%). No children had systemic symptoms or significant cervical lymphadenopathy. In contrast, tonsillar enlargement was observed to occur within a 6-week period in all children with TL, and 6 (86%) children had symptoms at presentation that included dysphagia in 5 (71%), snoring in 3 (43%), night sweats in 2 (29%), and fever and rigors in 2 (29%). Cervical lymphadenopathy greater than 3 cm was present in 6 (86%) children, while 1 child (14%) had hepatosplenomegaly. There was no histopathologic evidence of neoplasia in the UTE group, and a true discrepancy in size between the two tonsils was confirmed in only 21 of 44 (48%) cases. All 7 patients in the TL group had non-Hodgkin's lymphoma. All received chemotherapy, with 5 of the 7 cured and 2 dying of disease. The data suggest that tonsillectomy should be performed for biopsy purposes in UTE where there is a history of progressive enlargement, significant upper aerodigestive tract symptoms, systemic symptoms, suspicious appearance of the tonsil, cervical lymphadenopathy, or hepatosplenomegaly. The diagnosis of TL should also be considered when UTE is present in an immunocompromised child or one with a previous malignancy, when acute tonsillitis is asymmetric and unresponsive to medical treatment, or when rapid bilateral tonsil enlargement occurs. Observation is appropriate management for other cases of UTE.

KEY WORDS — tonsillar lymphoma, unilateral tonsillar enlargement.

INTRODUCTION

Unilateral tonsillar enlargement (UTE) or asymmetry of the size or appearance of the tonsils in the absence of acute infection is a relatively common finding in children and is often discovered incidentally. While tonsillar lymphoma (TL) and other malignancies of the tonsils are rare in children, the presence of UTE does raise this possibility and may thereby create significant anxiety for the parent and child, and occasionally also the treating physician.

Unilateral tonsillar enlargement in children may be an apparent rather than a true enlargement and be due to one tonsil's sitting in a more medial position in the tonsillar fossa relative to the other tonsil. This can be confirmed clinically by making the child gag to highlight the outline of the tonsils deep to the anterior tonsillar pillars and allow direct comparison of the size of the two tonsils. The tonsil can also be displaced medially by a peritonsillar abscess or a parapharyngeal mass.

There is little discussion in the medical literature regarding causes of UTE.[1] A summary of the causes of UTE in children is listed in Table 1. Acute infective causes include streptococcal tonsillitis with or without associated peritonsillar cellulitis or abscess. Vincent's angina and diphtheria are rare. Viruses include Epstein-Barr virus, cytomegalovirus, and a variety of less specific viral infections responsible for pharyngotonsillitis that may be asymmetric, such as myxovirus, picornavirus, respiratory syncytial virus, and reovirus.[1]

Chronic infections to be considered include tubercular tonsillitis, actinomycosis, and congenital syphilis.[1] Tonsillar hypertrophy, which may occur in re-

TABLE 1. CAUSES OF UNILATERAL TONSILLAR ENLARGEMENT IN CHILDREN

- Apparent enlargement
- True enlargement
  - Non-neoplastic
    - Acute infective
      - Bacterial
      - Viral
    - Chronic infective
    - Hypertrophy
    - Congenital
  - Neoplastic
    - Benign
    - Malignant

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TABLE 2. PRESENTING SYMPTOMS AND SIGNS IN UTE AND TL GROUPS OF PATIENTS

	UTE		*TL*	
Symptoms and Signs	*No.*	*%*	*No.*	*%*
Asymptomatic	26	57	1	14
Symptomatic	20	43	6	86
Local symptoms				
Recurrent sore throat	10	22	0	0
Snoring	5	11	3	43
Nasal obstruction	4	9	0	0
Dysphagia	1	2	5	71
Systemic symptoms				
Night sweats	0	0	2	29
Fever and rigors	0	0	2	29
Cervical lymphadenopathy >3 cm	0	0	6	86
Hepatosplenomegaly	0	0	1	14

UTE — unilateral tonsillar enlargement, TL — tonsillar lymphoma.

sponse to repeated infections, is frequently unilateral. Congenital tonsillar masses include teratoma, which has been well described,[2] hemangioma, lymphangioma, and cystic hygroma.

Benign neoplasms of the tonsil, which are relatively rare, were discussed by Hyams[3] in his review of 1,916 cases of tonsillar neoplasms. Most malignant neoplasms of the tonsil in children are lymphomas,[4,5] while squamous cell carcinoma, which is responsible for 85% to 90% of tonsillar malignancies in adults,[1] is rare in the pediatric age group.

Lymphomas of Waldeyer's ring are typically non-Hodgkin's lymphomas.[6,7] The 10 children with TL under 10 years of age reported by Hyams[3] included 9 with non-Hodgkin's lymphoma and 1 with Hodgkin's lymphoma. Most of these lymphomas were B-cell in origin. Shima et al[8] found that 38 of 43 Waldeyer's ring lymphomas in which immunophenotyping was performed were identified as being B-cell in origin, while Chan et al[9] found all of their 20 cases of Waldeyer's ring lymphomas outside the nasopharynx to express the B-cell phenotype.

In managing a child with UTE, the risks associated with unnecessary tonsillectomy must be balanced against those of delayed diagnosis of a TL. To help identify those children who require early biopsy, we reviewed the experience at our institution of children with UTE who underwent tonsillectomy for biopsy purposes over a 21-year period and compared the clinical presentation and the surgical and pathological findings with those of children who received treatment for TL in our institution over the same period of time.

METHODS

A chart review was carried out of 46 children with a diagnosis of UTE who were admitted to the Otolaryngology Department for biopsy tonsillectomy at the Royal Children's Hospital, Melbourne, Australia, between January 1, 1975, and December 31, 1995. The charts of 7 children who underwent treatment at our institution for TL over the same period of time, without necessarily having their initial biopsy at the Royal Children's Hospital, were also reviewed. Information was extracted regarding clinical presentation, laboratory investigations, surgical findings, histopathologic results, and outcome in the two groups of patients.

RESULTS

The age range of the 46 (26 male, 20 female) children in the UTE group was 2 to 13 years (mean 6.5), and that in the 7 (5 male, 2 female) children with TL was 2 to 9 years (mean 4.8).

The presenting symptoms and signs of the UTE and TL groups of patients are listed in Table 2. All children with TL were noted to have rapid tonsillar enlargement that developed over 2 to 6 weeks, and their presentation was characterized by local and systemic symptoms and marked cervical lymphadenopathy. All underwent biopsy soon after their initial presentation.

Eleven children from the UTE group were initially observed for a period ranging between 2 and 24 months (mean 6.6) prior to their surgery; however, the other 35 were scheduled for surgery at their first presentation. None of those in the UTE group had a history of rapid tonsillar enlargement, and only 20 (43%) were symptomatic. No child had systemic symptoms or cervical lymph node enlargement to greater than 3 cm in size.

All children with TL and 35 of those with UTE underwent laboratory investigations that included full blood count, erythrocyte sedimentation rate, and urea, electrolyte, and liver function tests. Two children with UTE had mild normochromic anemia (hemoglobin 99 and 101 g/L, normal range 115 to 135). Three children with TL had abnormal liver function test results (gamma glutamyltransferase 75, 200, and 240 U/L in the 3 children, normal range 0 to 40; and aspartate amino-transferase 65 and 90 U/L in 2 children, normal range 10 to 45), and 3 had elevated erythrocyte sedimentation rates (45, 60, and 70 mm/h, normal range 0 to 6). Chest x-rays, performed in all 7 children with TL, showed bilateral hilar lymphadenopathy in 3.

The surgical procedures performed are shown in Table 3. A comparative measurement of the size of the tonsils was made by the pathologist when bilateral tonsillectomy was performed. There was a significant difference in size in all 4 cases of TL, but only in 21 of 44 (48%) cases of UTE. The histopathologic diagnosis in the UTE group was chronic tonsillitis

TABLE 3. SURGICAL PROCEDURES PERFORMED IN UTE AND TL GROUPS OF PATIENTS

Surgery	*UTE*	*TL*
Tonsil biopsy	1	2
Unilateral tonsillectomy	1	1
Bilateral tonsillectomy	23	4
Adenotonsillectomy	21	0
Cervical lymph node biopsy in addition	0	6

in 32 children, reactive lymphoid hyperplasia in 10, and acute tonsillitis in 4. All 7 children in the TL group had non-Hodgkin's lymphoma, which included 1 Burkitt's lymphoma, 2 B-cell lymphomas, and 4 lymphomas in which immunophenotyping was not carried out.

Three children in the UTE group required readmission: 2 (4.3%) with secondary hemorrhage that settled spontaneously and 1 (2.2%) with dehydration. There were no postoperative complications in the TL group; however, 2 children went on to develop distant relapse and died despite further treatment. The remaining 5 are alive and well.

DISCUSSION

The typical presentation of TL in our study was one of rapid unilateral enlargement of the tonsil, often associated with local and systemic symptoms and cervical lymphadenopathy. This presentation resembles that of acute infective UTE, and suggests that the diagnosis of TL should be considered particularly in the child who presents clinically with acute tonsillitis where there is asymmetric tonsillar enlargement. Laboratory investigations may not help to differentiate between infection and lymphoma, but a chest x-ray may indicate a diagnosis of TL if hilar lymphadenopathy is detected.

In contrast, we found no cases of TL among the 46 children in whom UTE was long-standing and not associated with systemic symptoms or cervical lymphadenopathy. Suspicion of TL in this clinical setting is very low, and initial management by tonsillectomy for biopsy purposes is not warranted. It may, however, be difficult at the first presentation to determine whether UTE is long-standing or of recent onset. Similarly, a true discrepancy in size between the two tonsils was only found in 48% of the UTE group; this percentage highlights the importance of careful clinical assessment to differentiate between a unilaterally enlarged tonsil and one that is sitting in a more medial position in the tonsillar fossa relative to the other tonsil. Unnecessary tonsillectomy can thereby be avoided.

Ridgway et al[10] reported 5 cases of TL in children between 2 and 9 years old over a 13-year period in whom the diagnosis of TL was not necessarily suspected at the time of tonsillectomy. Tonsillectomy was performed in 1 child because of progressive bilateral tonsillar enlargement without asymmetry, and the child subsequently developed increasing cervical lymphadenopathy. The other 4 children all had UTE. One presented with a peritonsillar abscess that discharged spontaneously, while 3 had a short history of progressive UTE. Of these 3 children, 1 was asymptomatic, 1 had dysphagia, and 1 had cervical lymphadenopathy. In this series of children, the diagnosis of lymphoma was not suspected preoperatively. Ridgway et al[10] therefore advocated a detailed pathological review of all adenotonsillectomy specimens. The more diverse clinical presentation of TL among the group of patients reported by Ridgway et al differs from our experience, and underscores the degree of vigilance required to make an early diagnosis of TL.

The diagnosis of TL may not always be readily apparent from the appearance of the tonsil; however, an abnormal appearance is highly suspicious. Saul and Kapadia[11] described the tonsil in TL as gray-tan in color and of firm consistency. Other suspicious features included extension into adjacent soft tissue, an exophytic or polypoidal appearance, and the presence of ulceration.

Tonsillar lymphoma may be associated with bilateral symmetric enlargement of the tonsils, as seen in 1 of the cases cited by Ridgway et al.[10] Bilateral involvement in TL has been reported to range from 9% to 22%.[11]

In our series, TL developed in previously healthy children. It has been reported to occur as a second malignancy,[12] and in association with immunosuppression following bone marrow[13] and heart transplantation.[14] Abnormalities of the tonsils should clearly be regarded with great suspicion in the transplant population.

Tonsillectomy is recommended for biopsy purposes in UTE when there is a history of progressive enlargement or significant upper aerodigestive tract symptoms, particularly dysphagia; when there are systemic symptoms; when the appearance of the tonsil is suspicious; in the presence of cervical lymphadenopathy or hepatosplenomegaly; or when the child is immunocompromised or has a history of previous malignancy. The diagnosis of TL should also be considered particularly when acute tonsillitis is asymmetric and tonsillar asymmetry persists despite appropriate medical treatment, or when rapid bilateral tonsillar enlargement occurs. Observation initially is appropriate management for all other cases of UTE in children.

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REFERENCES

1. Cortez EA, Mattox DE, Holt GR, Gates GA. Unilateral tonsillar enlargement. Otolaryngol Head Neck Surg 1979;87: 707-16.

2. Shah BL, Vasan U, Raye JR. Teratoma of the tonsil in a premature infant. Case report and review of the literature. Am J Dis Child 1979;133:79-80.

3. Hyams VJ. Differential diagnosis of neoplasia of the palatine tonsil. Clin Otolaryngol 1978;3:117-26.

4. Paulsen J, Lennert K. Low-grade B-cell lymphoma of mucosa-associated lymphoid tissue type in Waldeyer's ring. Histopathology 1994;24:1-11.

5. Menarguez J, Mollejo M, Carrion R, et al. Waldeyer ring lymphomas. A clinicopathological study of 79 cases. Histopathology 1994;24:13-22.

6. Todd GB, Michaels L. Hodgkin's disease involving Waldeyer's lymphoid ring. Cancer 1974;34:1769-78.

7. McNelis FL, Pai VT. Malignant lymphoma of head and neck. Laryngoscope 1969;79:1076-87.

8. Shima N, Kobashi Y, Tsutsui K, et al. Extranodal non-Hodgkin's lymphoma of the head and neck. A clinicopathologic study in the Koyoto-nara region of Japan. Cancer 1990;66:1190-7.

9. Chan JK, Ng CS, Lo ST. Immunohistological characterization of malignant lymphomas of the Waldeyer's ring other than the nasopharynx. Histopathology 1987;11:885-99.

10. Ridgway D, Wolff LJ, Neerhout RC, Tilford DL. Unsuspected non-Hodgkin's lymphoma of the tonsils and adenoids in children. Pediatrics 1987;79:399-402.

11. Saul SH, Kapadia SB. Primary lymphoma of Waldeyer's ring. Clinicopathologic study of 68 cases. Cancer 1985;56:157-66.

12. Coppes MJ, Byard R, Stringer DA, McLorie GA, Silver MM, Solh H. T-cell lymphoma and mature nephroblastoma after syndromous bilateral Wilms' tumor. Cancer 1991;67:337-41.

13. Yellin SA, Weiss MH, Kraus DH, Papadopoulos EB. Tonsil lymphoma presenting as tonsillitis after bone marrow transplantation. Otolaryngol Head Neck Surg 1995;112:544-8.

14. Fairley JW, Hunt BJ, Glover GW, Radley-Smith R-C, Yacoub MH. Unusual lymphoproliferative oropharyngeal lesions in heart and heart-lung transplant recipients. J Laryngol Otol 1990;104:720-4.

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INTERNATIONAL CONFERENCE ON NEWBORN HEARING SCREENING DIAGNOSIS AND INTERVENTION

The International Conference on Newborn Hearing Screening Diagnosis and Intervention will be held in Milan, Italy, October 12-14, 2000. In Europe, contact Dr Ferdinando Grandori, Center of Biomedical Engineering, Polytechnic of Milan, piazza Leonardo da Vinci 32, I-20133 Milan, Italy; fax +39.02.2399.3360; e-mail NHS2000@biomed.polimi.it. In the United States, contact Dr Deborah Hayes, The Children's Hospital, 1056 E 19th Ave, B030, Denver, CO 80218; telephone (303) 861-6424; fax (303) 764-8220; e-mail: Hayes.deborah@tchden.org.

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MANAGEMENT OF PERSISTENT TRACHEOCUTANEOUS FISTULA IN THE PEDIATRIC AGE GROUP

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Different surgical methods have been advocated for closure of persistent tracheocutaneous fistula (TCF) in children. The objective of this study was to compare different methods of repair and postoperative care that were used for management of TCF in children. The charts of 98 children with persistent TCF who were surgically managed in our department between January 1990 and April 1997 were reviewed retrospectively. Excision of the fistulous tract and healing by secondary intention was employed in 18 patients. Eighty patients were managed by tract excision followed by primary closure. Sixty-three patients remained intubated for 18 to 24 hours postoperatively, while 17 patients were extubated in the recovery room. One patient had a large tracheal granuloma on follow-up endoscopy. Three patients needed a second procedure. No significant correlation was found between the method of surgical repair or the length of postoperative intubation and outcome. In our experience, TCF repair, either by primary closure or secondary intention, is a relatively safe and effective procedure in the pediatric age group. Preoperative evaluation and possible indications for selecting the method of repair are discussed.

KEY WORDS — pediatrics, tracheocutaneous fistula, tracheotomy.

INTRODUCTION

Tracheocutaneous fistula (TCF) is one of the recognized sequelae of tracheotomy in the pediatric age group.[1] It occurs secondary to epithelial growth into the tracheotomy tract that prevents closure and healing (Fig 1A). The reported incidence of TCF ranges widely, from 3.3%[2] to 43%.[3] Persistence of TCF after decannulation appears to depend on the length of cannulation time.[3,4] The higher incidence of TCF in children in recent reports may reflect the change in the indications for pediatric tracheotomy over the last 2 decades.[5,6] Very few tracheotomies are now performed for acute inflammatory conditions with a short cannulation time, while there has been an increase in the proportion of pediatric tracheotomies performed for subglottic stenosis with a longer cannulation time.

Persistence of TCF may cause considerable morbidity due to recurrent aspiration and subsequent respiratory infection, difficulty in phonation, ineffective cough, skin irritation, cosmetic objection, social inacceptance, and intolerance to submersion. Therefore, surgical intervention is indicated. Several surgical techniques have been described. However, authors' recommendations for surgical repair of TCF are often based on small numbers of isolated cases, and the best surgical approach is still controversial.

We report our experience with TCF repair in 98 pediatric patients submitted to different methods of surgery and postoperative care.

MATERIALS AND METHODS

A retrospective review was undertaken for all the pediatric patients (under 18 years) who underwent surgical repair of TCF in our department between January 1990 and April 1997. The patients' charts were analyzed with respect to the following variables: indication for tracheotomy, age at the time of tracheotomy, lengths of cannulation and decannulation, endoscopic findings, associated medical problems at the time of TCF repair, method of surgical repair, postoperative intubation, early and late complications, and final result.

Two principal methods of surgical repair were used for the duration of the study. The first method included fistulectomy with primary closure. An elliptical skin incision was made around the fistula, and the skin above and below the incision was widely undermined. Dissection was carried down through scarred skin and subcutaneous tissue around the fistula to the trachea (Fig 1B). After complete mobilization of

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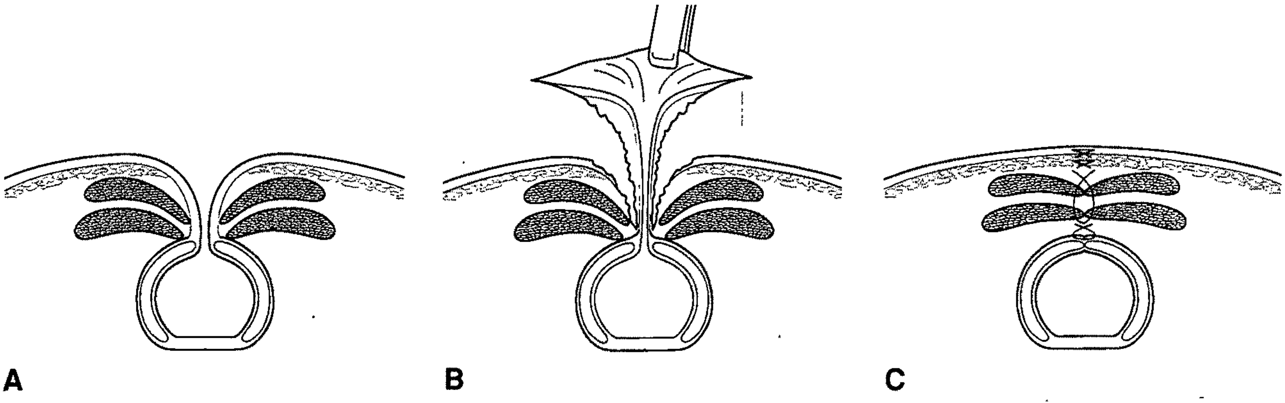

Fig 1. Technique of tracheocutaneous fistula closure. A) Ingrowth of squamous epithelium along tracheocutaneous tract. B) Tracheocutaneous tract dissected circumferentially down to trachea. C) Multilayer closure after fistulectomy.

the tract, it was resected from the trachea, and the edges were approximated with absorbable sutures. The strap muscles, which were often incorporated in the scar tissue or adherent to the trachea, were freed, repositioned over the trachea, and closed over a small rubber band drain. Subcutaneous tissue was closed and the skin sutured (Fig 1C). Postoperatively, the patients were either extubated in the recovery room, when fully awake, or kept intubated for 18 to 24 hours. The decision regarding the length of postoperative intubation was made according to the attending otolaryngologist's preference. The second method of TCF repair involved excision of the fistulous tract, placement of a 3-mm tracheotomy tube that was removed after 12 to 24 hours, and healing by secondary intention.

All patients underwent direct laryngoscopy and bronchoscopy at the time of TCF repair, to rule out any airway obstruction, and about 1 month after the repair, to exclude formation of granulation tissue or stricture at the previous tracheotomy site.

Kendall nonparametric correlation coefficients were used to calculate correlation between the preoperative variables (indication for tracheotomy, age, length of cannulation and decannulation, associated medical problems), methods of surgical repair, and duration of postoperative intubation and the outcome (early and late complications, closure of TCF). A p value of <.05 was considered significant.

TABLE 1. INDICATIONS FOR TRACHEOTOMY

Indication	*Patients (N = 98)*
Subglottic stenosis	42
Long-term ventilation	25
Tracheomalacia	10
Bilateral vocal cord paralysis	6
Pierre Robin syndrome	6
Cervical cystic hygroma	3
Laryngeal cleft	2
Laryngomalacia	2
Respiratory papillomatosis	1
Base of tongue cyst	1

RESULTS

One hundred four pediatric patients underwent TCF repair in our department during the defined period. Six patients were excluded from the study due to insufficient information in their charts. The study group included 60 boys and 38 girls. The mean age at the time of tracheotomy was 24 months (range 1 day to 109 months). Subglottic stenosis was the most common indication for tracheotomy (Table 1). The mean durations of cannulation and decannulation were 41.9 and 8.3 months, respectively. The mean age at the time of TCF repair was 74.2 months (range 19 to 200 months). Fourteen patients had associated medical disorders at the time of TCF repair (Table 2). Eighty patients were managed by fistulectomy and primary closure. Sixty-three patients were extubated 18 to 24 hours after the operation, while 17 patients were extubated in the recovery room. Excision of the fistulous tract and healing by secondary intention was employed in 18 patients. One patient displayed acute airway distress following primary repair and was recannulated. In 1 patient, a large tracheal granuloma at the previous tracheotomy site was found on follow-up endoscopy (Fig 2). It was removed endoscopically without difficulty. There were no other early or late complications. In the patients

TABLE 2. ASSOCIATED MEDICAL DISORDERS

Disorder	*Patients (N = 14)*
Bronchopulmonary dysplasia	4
Cardiac disease	3
Epilepsy	2
Down syndrome	2
Cerebral palsy	2
Hydrocephalus	1

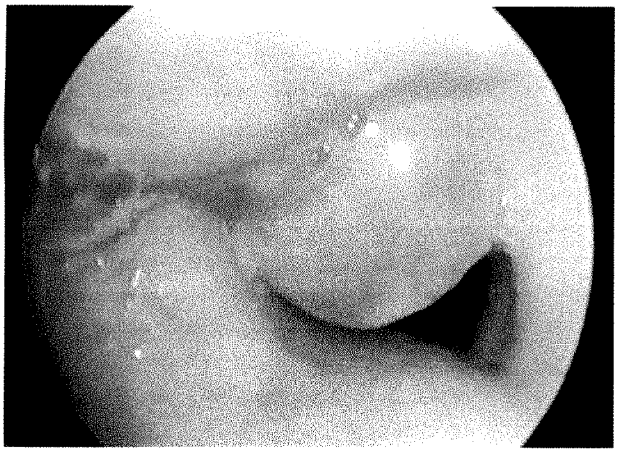

Fig 2. Tracheal granuloma at previous tracheocutaneous fistula site.

who underwent excision of the fistulous tract and healing by secondary intention, closure of the TCF was noted after 4 weeks in 15 patients and after 8 weeks in 2 patients. In 3 patients, closure of the TCF was not achieved after the initial procedure (2 primary closures and 1 by secondary intention). These patients were successfully managed by a second procedure. No significant correlation was found between the preoperative variables, method of repair, or duration of postoperative intubation and the outcome. This lack of statistical significance may be due to the low number of complications and unsuccessful repairs in our series.

DISCUSSION

Tracheocutaneous fistula is a relatively frequent sequela of tracheotomy in the pediatric age group. Therefore, decannulation by routine excision of the tracheostomy tract followed by either short-term recannulation[7] or closure of the tracheotomy[8] has been recommended. However, usually a period of observation after the decannulation is appropriate. If the TCF persists, definitive treatment is necessary. Several methods of surgical repair have been described: primary closure in layers,[9] fistulectomy and primary closure in layers,[9-11] and fistulectomy and healing by secondary intention.[12]

The major concern regarding primary TCF closure is that air leaking from the trachea will track along subcutaneous tissue planes into the mediastinum and pleural cavity, causing subcutaneous emphysema, pneumomediastinum, and pneumothorax. Several precautions can be taken in order to decrease the risk of these complications. Saline irrigation of the wound with positive pressure ventilation can be used to check for significant air leakage before closure of the strap muscles. Extubating the patients in the recovery room when they are fully awake may assist in avoiding paroxysmal coughing during recovery from the anesthesia. Overnight intubation may allow closure of the tracheal defect. However, a longer intubation period may be associated with possible laryngeal injury (especially in patients who underwent previous laryngotracheal reconstruction), increased morbidity, and higher hospital costs.

Insertion of an effective drain will allow a route for air to escape via the skin. Indeed, several complications due to air dissection into the neck and chest were reported in children who underwent TCF with primary closure and without a drain.[13] However, this complication was not noted when the surgical technique included closure over a drain.[11] Laryngeal or suprastomal obstruction at the time of the repair will also cause increased airway pressures. Therefore, careful endoscopic evaluation of the airway just prior to the TCF repair is of great importance.

Fistulectomy with closure by secondary intention was advocated to avoid problems of air tracking postoperatively.[12,14] However, these recommendations were based on a small number of patients, and the authors did not mention whether insertion of a drain was part of the primary closure. Healing by secondary intention may result in delayed wound healing, unpredictable scar appearance, and less acceptable cosmesis. Moreover, the need for short recannulation may cause an undesirable emotional impact on the patient and his or her family. This method of repair should be considered when the tracheal opening of the fistula is large. It may also be suitable for children with lung disease, since they appear at increased risk of developing complications after primary closure due to frequent cough and high transmural airway pressures with forceful coughing. Another possible indication for closure by secondary intention may be a variable or unpredictable degree of suprastomal obstruction such as obstruction due to bilateral vocal cord paralysis.

CONCLUSION

In our experience, TCF repair, either by primary closure with insertion of a drain or by secondary intention, is a relatively safe and effective technique in the pediatric age group. However, although this series had good outcomes overall, by the nature of its design, ie, a retrospective case series, it is mainly descriptive, and no definitive conclusions of causality can be drawn. Short postoperative intubation with extubation in the recovery room was not associated with a higher complication rate compared to a longer intubation period. Therefore, short-term postoperative intubations may be sufficient. Primary closure offers a decreased recovery period and improved cosmesis. We believe that primary closure and extubation in the recovery room should be considered as the preferred method for management of persistent TCF. Fistulectomy and healing by secondary intention should be considered if the tracheal defect is large or in children with compromised pulmonary function. Careful endoscopic evaluation of the airway should be employed to rule out airway obstruction.

REFERENCES

1. Myers EN, Stool SE. Complications of tracheotomy. In: Myers EN, Stool SE, Johnson J, eds. Tracheotomy. New York, NY: Churchill Livingstone, 1985:147-69.
2. Oliver P, Richardson JR, Clubb RW, Flake CG. Tracheotomy in children. N Engl J Med 1962;267:631-7.
3. Joseph HT, Toni P, Preece JM, Bailey CM, Evans JNG. Pediatric tracheostomy: persistent tracheocutaneous fistula following decannulation. Int J Pediatr Otorhinolaryngol 1991;22:231-6.
4. Wetmore RF, Handler SD, Potsic WP. Pediatric tracheostomy. Experience during the past decade. Ann Otol Rhinol Laryngol 1982;91:628-32.
5. Arcand P, Granger J. Pediatric tracheostomies: changing trends. J Otolaryngol 1988;17:121-4.
6. Swift AC, Rogers JH. The changing indications for tracheostomy in children. J Laryngol Otol 1987;101:7258-62.
7. Friedberg J. Tracheotomy revision in infants: to facilitate extubation and restore phonation. Int J Pediatr Otorhinolaryngol 1987;13:61-8.
8. Rogers JH. Decannulation by external exploration of the tracheostomy in children. J Laryngol Otol 1980;94:563-7.
9. Bishop JB, Bostwick J, Nekoi F. Persistent tracheal stoma. Am J Surg 1980;140:709-10.
10. Haynes JH, Bagwell CE, Salzberg AM. Management of persistent pediatric tracheostomal fistulas. J Pediatr Surg 1995;30:566-7.
11. Bressler KL, Kaiser PC, Dunham ME, Holinger LD. Primary closure of persistent tracheocutaneous fistula in children. Ann Otol Rhinol Laryngol 1994;103:835-7.
12. Keenan JP, Snyder GG, Lehmann WB, Ruiz JW. Management of tracheocutaneous fistula. Arch Otolaryngol 1978;104:530-1.
13. Wheeler WB, Kuracheck SC, Lobas JG, Lipscomb TS. Respiratory complications of tracheocutaneous fistula closure. Crit Care Med 1991;14:580-2.
14. White AK, Smitheringale AJ. Treatment of tracheocutaneous fistula in children. J Otolaryngol 1984;18:49-52.

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OTALGIA AND AVERSIVE SYMPTOMS IN TEMPOROMANDIBULAR DISORDERS

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The term Costen's syndrome has been used in the dentomedical literature to describe a constellation of craniofacial symptoms. Since some of the same complaints have been reported in patients with "generalized" psychological distress, symptoms associated with the syndrome may not be useful in differential diagnosis of temporomandibular disorders. The present study investigated whether some somatic complaints, particularly tinnitus and dizziness, were pathognomonic in patients with chronic temporomandibular pain. Illness behavior and personality factors were studied for possible interrelationships with these symptoms. Factor analysis revealed that tinnitus and dizziness loaded on separate factors. Tinnitus loaded with nasal stuffiness, tearing, and itching of the eyelids and nose, while dizziness loaded with complaints of altered taste and smell and blurred vision. Neither was consistently related to measures of pain or to indices of illness behavior or somatic focus.

KEY WORDS — Costen's syndrome, dizziness, Meniere's disease, psychological disorder, temporomandibular disorder, tinnitus.

INTRODUCTION

Temporomandibular disorders (TMDs) include musculoskeletal complaints that represent a family of clinical conditions.[1] The cardinal symptoms and signs are pain in or surrounding the temporomandibular joint (TMJ), limitation on mandibular opening, tenderness on palpation of the masticatory muscles or TMJs, and joint sounds. Because other, nonspecific symptoms may frequently coexist, there is difficulty in deciding if they should be included in the diagnosis.

Part of our understanding of TMDs derives from what has been termed in the dentomedical literature as Costen's syndrome. The original description was of a constellation of symptoms including otalgia, tinnitus, impaired hearing, and aural congestion.[2,3] The dysfunction and associated symptoms were attributed to retroposition of the mandibular condyle and mandibular overclosure that compresses the auriculotemporal or chorda tympani nerve. Presumably, the compression irritates the eustachian tube and changes the intratympanic pressure, leading to ear symptoms.[4]

Another line of thought was that ear symptoms resulted from the condyle's pressing against the anterior tympanic artery and the chorda tympani at the petrotympanic fissure.[5] Anatomic dissection of the temporomandibular region failed to confirm that the condyle exerted pressure on these tissues.[6-8] Certain modern practitioners endorse the basic philosophy of Costen's syndrome for patients suffering symptoms.[9] The position is that occlusal dysharmonies produce condylar displacement leading to auditory and vestibular symptoms such as tinnitus and dizziness.

Similar symptoms have been reported in patients suffering cervicogenic complaints. Several studies relate soreness of the neck to myofascial[10-13] or TMJ[14] pain and associate tinnitus and dizziness with these problems. Barre-Lieou syndrome, first described in 1926, includes symptoms virtually identical to those of Costen's. The cause has been attributed to posttraumatic disk prolapse at the cervical 3 and 4 levels.[15] Symptoms common to both syndromes have been reported in patients suffering bony spinal disorders[16] and late whiplash syndrome.[17] Dysfunction of the atlanto-occipital joint has been implicated in the frequent combination of facial pain, TMJ pain, and headache.[18] Such symptoms have been attributed to vascular compression of cervical 2 and 3 nerve roots, producing an inadequate flow of blood in the vertebrobasilar system.[19] Another argument is that disk prolapse of cervical vertebrae 3 and 4 causes dysfunction of the craniocervical sympathetic chain and surrounding vertebral vessels.[20]

The presence of dizziness has been interpreted variously. A dysfunctional relationship between neck proprioceptive centers with feedback via the brain stem and interference with labyrinthine function has been proposed.[21] Dizziness, TMJ crepitus, excessive

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lacrimation, blurred vision, photophobia, and phonophobia have been mentioned in patients suffering from cervicogenic headache.[22] The authors hypothesized that head movement or pressure on muscular trigger points in the dorsal cervical musculature leads to headache. These muscles form part of the proprioceptive system interconnecting with the vestibular nuclei of the spinovestibular tract and with the nuclei of the extraocular muscles by the medial longitudinal fasciculi. Thus, hyperactive cervical muscles might provoke an unsteady gate with concomitant dizziness.

Some symptoms associated with TMD have been attributed to dysfunction of the inner ear. In 1861, Prosper Meniere described lesions specific to the inner ear leading to tinnitus, dizziness, and deafness.[23,24] Many clinicians have attributed these symptoms to recurrent endolymphatic hydrops.[25,26] Magnetic resonance imaging studies have confirmed that recurrent ruptures of the membranous labyrinth are the primary cause of increased endolymphatic volume.[25] An imbalance between secretion and resorption of endolymph is assumed to obstruct the duct and sac. A recent review of the literature discredits this central dogma of a relationship between hydrops and these symptoms.[26]

Psychological factors have also been implicated in symptoms frequently associated with TMD. Dizziness and tinnitus, in particular, have been reported in patients suffering from anxiety,[27,28] mood disorders,[28] depression,[29-31] and "generalized" psychological distress.[32] Orofacial pain has been described as coexisting with dizziness and tinnitus in 17% of depressed patients[30] and present in one third of nonneuralgiform pain patients with generalized emotional problems.[32]

Since the triad of Costen's symptoms, namely, tinnitus, TMD pain, and dizziness, is common among different disorders, the present study evaluated the possible coexistence of these symptoms along with other somatic complaints commonly reported by patients suffering TMD pain. We were particularly interested in possible interrelationships among these symptoms, as well as their relation to illness behavior and personality traits.

METHODS

A sample of 99 consecutive admissions to the Virginia Commonwealth University Temporomandibular Joint–Orofacial Pain Center was the study population. The Center is a regional clinic for referral of patients with various kinds of facial pain. Thus, the severity of physical complaints seen in this population may be greater than would be expected in natural populations.

The mean age of the population was 36 (±12.0 SD) years. Eighty-six percent were women, with approximately 60% of the population having a minimum of 12 years of school. The mean duration of pain was 3.5 (±5.2 SD) years.

Under the supervision of a trained clinician, all patients completed a consent form and health questionnaire that included questions about the nature of their complaints. Details of this questionnaire have been published elsewhere.[33,34]

Patients completed the Visual Analog Scale (VAS) for pain[35] and emotions,[35] the McGill Pain Questionnaire (MPQ),[36] the Pilowsky Illness Behavior Questionnaire (IBQ),[37] and the Minnesota Multiphasic Personality Inventory (MMPI).[38]

The patients' responses to questions concerning their symptoms were subjected to unweighted least square factoring using varimax rotation. Details of this analysis have been described previously.[39,40] This common factor analysis represents a family of techniques used to analyze the variation in a set of data and classify it according to 2 sources: the common factors and the unique factors estimated only from the variation due to the common factors.[41]

Diagnoses were based upon a complete history and clinical evaluation, including radiologic analysis, measurement of mandibular limitation on motion, digital palpation of the TMJ and masticatory muscles, and recording of joint sounds. A detailed description of these procedures has been published elsewhere.[33] The same examiner (F.M.B.) performed all examinations.

Approximately 59% of the patients suffered from primarily myogenous pain surrounding the TMJ.[34] The next most common pain was arthrogenous in nature: 17% had primarily disk disorders and 4% had osteoarthritis of the TMJ. Another 20% were patients suffering from idiopathic facial pain.

RESULTS

Principal components factor analysis using varimax rotation showed that the symptoms (Table 1) are well represented by 3 factors. Symptoms of factor 1 involved functions of cranial nerves I, II, III, and VII and included dizziness, blurred vision, altered taste, and altered smell. Factor 2 appeared to describe mucosal-otic functions, including tinnitus, nasal stuffiness, tearing of the eyes, and itching of the eyelids, nose, and throat. Factor 3 was interpreted as being related to sympathetically mediated hyperactivity, primarily sweating.

TABLE 1. FACTOR LOADINGS FOR PRINCIPAL COMPONENT ANALYSIS (VARIMAX ROTATION) OF NONPAINFUL SYMPTOMS

Measure	*Factor 1 (Cranial)*	*Factor 2 (Mucosal-Otic)*	*Factor 3 (Sympathetically Mediated)*
Altered taste	0.816		
Blurred vision	0.747		0.244
Altered smell	0.718	0.314	
Dizziness	0.656		
Itching (eyelids, nose)		0.819	
Excessive tearing		0.695	0.202
Tinnitus	0.358	0.596	
Nasal stuffiness	0.380	0.594	
Excessive sweating of one body area			0.747
Excessive sweating of whole body	0.298		0.713
Eigenvalue	3.3	1.4	1.2
Cumulative percent variance	32.6	46.5	58.5

The relation of the 3 factors to standardized measures of pain produced mixed results. None of the scales was consistently related to standardized measures of MPQ pain or pain ratings of the VAS (Table 2). Although each factor had 1 or more statistically significant results in relation to the pain intensity (sensory) VAS scores, the Pearson coefficients were low. The most consistent agreement between sensory and affective VAS pain scores occurred with symptoms of factor 3.

Only symptoms of factor 3 related to dimensions of the emotion VAS pain scales (Table 3). Reports of excessive sweating related to 4 of 5 dimensions and also were significantly related to general hypochondriasis on the IBQ and the MMPI (Table 3). All factors correlated with disease conviction on the IBQ. None of the remaining scales was consistently related to the symptoms.

DISCUSSION

The current factor analysis showed that dizziness and tinnitus loaded on different factors. The Pearson correlation coefficients were low for both symptoms with regard to pain. Symptoms assigned to cranial nerves (factor 1) showed a weak relationship to MPQ scales. Those of otic-mucosal (factor 2) origin were even less related, and those of sympathetically mediated (factor 3) origin were not at all related. This overall heterogeneity casts doubt on the commonality of these symptoms.

A survey of the literature reveals 38 studies of patients with possible commonality of these symptoms (Table 4[2,3,13,14,21,42-73]). The negative evidence against relationships includes aural symptoms to otal-

TABLE 2. COMPARISON OF THREE FACTORS AND STATISTICALLY SIGNIFICANT VAS SCALES FOR SENSORY AND AFFECTIVE LEVELS OF PAIN AND PEARSON CORRELATION COEFFICIENTS FOR DIFFERENT DIMENSIONS OF MPQ

Measure	*Factor 1 (Cranial)*	*Factor 2 (Mucosal-Otic)*	*Factor 3 (Sympathetically Mediated)*
VAS			
Sensory pain			
Low	0.259 (p = .02)	NS	NS
Usual	0.225 (p = .04)	NS	NS
High	NS	0.284 (p = .009)	0.277 (p = .01)
Affective pain			
Low	NS	NS	NS
Usual	NS	NS	0.270 (p = .02)
High	NS	0.252 (p = .02)	NS
MPQ			
Sensory	NS	0.201 (p = .05)	NS
Affective	0.287 (p = .005)	NS	NS
Evaluative	NS	NS	NS
Miscellaneous	0.415 (p = .0001)	NS	NS

VAS — Visual Analog Scale, MPQ — McGill Pain Questionnaire, NS — not statistically significant.

TABLE 3. COMPARISON OF THREE FACTORS AND STATISTICALLY SIGNIFICANT PEARSON COEFFICIENTS FOR VAS-EMOTIONS AND COMPONENTS OF IBQ AND MMPI

Measure	*Factor 1 (Cranial)*	*Factor 2 (Mucosal-Otic)*	*Factor 3 (Sympathetically Mediated)*
VAS-emotions			
Depression	NS	NS	0.315 (p = .004)
Anxiety	NS	NS	0.236 (p = .03)
Frustration	NS	NS	0.257 (p = .02)
Anger	NS	NS	NS
Fear	NS	NS	0.258 (p = .02)
MPQ			
General hypochondriasis	NS	NS	0.314 (p = .002)
Disease conviction	0.364 (p = .001)	0.257 (p = .01)	0.226 (p = .03)
Psychological versus somatic illness	NS	NS	NS
Affective inhibition	NS	NS	NS
Denial	NS	NS	NS
Irritability	NS	NS	NS
MMPI			
L scale	NS	NS	NS
F scale	NS	NS	NS
Hypochondriasis	NS	NS	0.451 (p = .004)
Depression	NS	NS	NS
Hysteria	0.415 (p = .008)	NS	0.470 (p = .002)
Psychopathic deviation	NS	NS	NS
Masculine/feminine	NS	NS	NS
Paranoia	NS	NS	NS
Psychasthenia	NS	NS	NS
Schizophrenia	NS	NS	NS
Hypomania	NS	NS	NS
Social introversion	NS	NS	NS

VAS — Visual Analog Scale, IBQ — Pilowsky Illness Behavior Questionnaire, MMPI — Minnesota Multiphasic Personality Inventory.

gia,[21] otalgia to tinnitus,[16] TMJ pain to tinnitus and to dizziness,[74] and TMJ and/or ear pain to neither tinnitus nor TMJ internal derangement.[71] Data of 1 TMD study[72] (Table 4) collected over 16 years were not tested statistically, but extrapolation reveals that approximately two thirds of TMD patients with TMJ pain lacked tinnitus and 60% lacked dizziness.[72] The Tinnitus Data Registry also confirmed that just 7% of patients with severe tinnitus had a history of TMJ complaints.[65] No single indicator separated patients with tinnitus from patients with tinnitus and TMD. Such heterogeneity in findings makes one question the existence of significant associations between these symptoms.

Positive associations have been reported: TMJ pain and muscle trigger point pain on palpation,[66] and tinnitus with otalgia and dizziness with otalgia in TMD cases.[70] The authors concluded that tinnitus, dizziness, and otalgia were significantly more prevalent in TMD patients than in 2 non-TMD samples.[70] They did not test the frequency of tinnitus versus the frequency of dizziness in TMD patients.

Our findings relating common symptoms of TMD with pain and emotion scales (VAS-emotions, IBQ, and MMPI) were inconsistent (Table 3). On the whole, the pattern of nonspecific symptoms mirrored that reported in other studies of seemingly unrelated disorders.[75-88] A similar conclusion can be drawn from other studies (Table 5[27-31,82,83,86,87]) of patients with different emotional disorders. Some patients are bothered emotionally; others are not. For example, responses made by tinnitus sufferers on coping scales showed that "low copers" to tinnitus were more psychologically distressed than "high copers."[88] The prevalence of dizziness proved greater in patients with high levels of depression when compared to patients with low levels of depression.[82] But the frequencies of tinnitus and dizziness were low in another group of depressed patients.[30] The finding of orofacial symptoms in some subjects and not in others raises serious doubt as to a singular disorder consistent with Costen's syndrome.

Various hypotheses have been proposed to explain an etiologic relationship between otolaryngological symptoms and TMD. Arguments for the occlusion hypothesis are weak in experimental, epidemiologi-

TABLE 4. PERCENT FREQUENCY OF SYMPTOMS IN DIFFERENT POPULATIONS

Orofacial Pain	*Tinnitus*	*Dizziness*	*No. of Patients*	*Reference*
38	13	12	400	Costen[2]
Part of 57	Part of 32	8	165	Costen[3]
3	14	5	91	Goodfriend[42]
13 Facial, 12 ear	1	0	82	Hankey[43]
43 TMJ, 13 ear	27	5	100	Gelb and Arnold[44]
Ear mostly	25	0	12	Capps[45]
13 Facial, 12 ear	9	0	68	Hankey[46]
47 Ear, 35 jaw, 15 TMJ	65	8	66	Dolowitz et al[47]
17	31	27	1,391	Myrhaug[48]
94 Total, 42 ear, 16 TMJ	1	0	150	Vero[49]
35 Ear, 15 preauricular, 5 facial	42	18	742	Gelb et al[50]
93 Total, 52 ear	42	0	86	Bernstein et al[51]
11 Ear, 82 on palpation	7	0	56	Zarb and Thompson[52]
52 TMJ	46	12	200	Gelb and Tarte[14]
100		*	42 TMD	Sharav et al[13]
0			35 Non-TMD	
100	44	44	25	Principato and Barwell[53]
82 Ear, 6 facial	4	4	45	Brookes et al[21]
47	19	0	47	Koskinen et al[54]
38 Jaw and/or face	15	17	47	Carlsson et al[55]
29 TMJ, 9 facial, 5 ear	1	0	200	Gelb and Bernstein[56]
68	19	0	31	Thornburn and Blake[57]
61	2	0	350	Wedel and Carlsson[58]
40	36	0	837	Cooper et al[59]
32 Jaw and/or face	14	15	148	Wedel and Carlsson[60]
35†	33	0	105	Bush[61]
38 Jaw and/or face	25	0	376	Rubinstein and Carlsson[62]
100	100	44	25	Williamson[63]
2 to 11	25	0	99	Rubinstein et al[64]
45	100	†	69 TMD	Vernon et al[65]
32	100		860 Non-TMD	
75* TMJ, 75* muscles	100	0	20	Morgan[66]
100	59	70	200	Chole and Parker[67]
51 TMJ, 44 facial	63	41	996 Clinic	Cooper and Cooper[68]
52 TMJ, 43 facial	46	38	1,764 Private	
17	0	13	126 Dental	Englund et al[69]
100	59	70	200 TMD	Parker and Chole[70]
10	14	30	302 Medical	
14	33	44	347 Dental hygiene	
98 TMJ and/or ear, 79 facial, 43 temporal, 38 back of eye	100	57	53 ID	Ren and Isberg[71]
92 TMJ and/or ear, 56 facial‡, 14 temporal§, 11 back of eye§	0	28	82 ID	
97	36	40	200 TMD	Gelb et al[72]
43	42	18	742 Ear-nose-throat	
32	46	12	200 Headache	
38	38	0	93 TMD	Wright and Bifano[73]

TMJ — temporomandibular joint, TMD — temporomandibular disorder.

*Pain judged by digital palpation of sternocleidomastoid muscle was significant, but not significant with 6 other muscles.

†No significant difference between groups or symptoms.

‡p = .01.

§p = .001 between group with disk displacement (internal derangement; ID) with tinnitus and ID without tinnitus.

cal, and clinical studies. Further, tinnitus sufferers do not recognize a relationship. Three fourths in 1 study responded “no” influence and another 13% “don’t know” to questions about a possible influ-

TABLE 5. PERCENT FREQUENCY OF PATIENTS WITH EMOTIONAL DISORDERS AND PHYSICAL SYMPTOMS IN DIFFERENT POPULATIONS

Disorder	*Tinnitus*	*Dizziness*	*Hearing Impairment*	*No. of Patients*	*Reference*
Anxiety	100	21	24	62	Hallam and Stephens[27]
Anxiety and depression	100	0	0	112	Halford and Anderson[87]
Anxiety	0	100	0	17	Simpson et al[28]
Mood	100	0	60	24	
Depression	8	13	0	48	Minnigerode and Harbrecht[30]
Depression	100	0	83	40 (tinnitus)	Sullivan et al[29]
	0	0	100	14 (nontinnitus)	
Depression	77	40	0	116 (20-39 y)	Koenig et al[31]
	66	47	0	332 (70-102 y)	
Depression					Waxman et al[82]
Low	0	3	0	36	
Medium	0	22	0	49	
High	0	32	0	42	
Depression	100	0	0	21	Harrop-Griffiths et al[86]
Emotional problems	100	14	53	72	Tyler and Baker[83]

ence of occlusal change on tinnitus.[64,85] Other etiologic hypotheses involve the tensor tympani, otomandibular ligament, eustachian tube, and otologic disease.[67,68] Argument for the ligament hypothesis depends on direct stimulation of the malleus by the diskomalleolar ligament and the mallear part of the sphenomandibular ligament. An argument against the ligament hypothesis is that the ligaments cannot transmit enough mechanical energy to the malleus to account for the occurrence of high-frequency subjective tinnitus and positional changes in the malleus producing dizziness.[70]

Another hypothesis is that blockage or congestion of the eustachian tube results from hyperactivity of the tensor veli palatini and leads to earache, tinnitus, and dizziness. But tubal resistance, a measure of tensor veli palatini function, was not significantly different between TMD and non-TMD patients.[21] Further, tympanometric studies have not sustained a relationship between TMD and aural symptoms.[21,89] Hearing loss was not greater in TMD patients with tinnitus versus patients with tinnitus attributed to other causes.[65] A positive relationship was mentioned between deafness and severe TMJ arthropathy.[74] Unfortunately, deafness was assessed by patient response to a questionnaire.

A retrocochlear origin has been suggested for tinnitus. Studies conducted with positron emission tomography showed that tinnitus patients had an ability to modulate tinnitus loudness by performing voluntary orofacial movements.[90] A widespread activation of tone and aberrant links between the auditory and limbic systems were found in tinnitus patients, but not in subjects with normal hearing. This change in quality of tinnitus had been previously associated with jaw movements.[64] In addition, other regions of the brain have been shown to be involved. Both tinnitus and dizziness, as well as facial pain and altered sensation of the trigeminal nerve, have been reported in patients with cerebellopontine angle meningiomas.[91]

Analysis of the extant literature reflects differences between statistical significance and clinical interpretations. Different health professions involved in the diagnosis of patients hold to various causes, ranging from emotional distress to mechanical malfunction. Such differing views have caused confusion, leading to different diagnoses and treatments for the constellation of symptoms and history associated with Costen's syndrome. Overemphasis on coexisting symptoms that may not be genuinely causally related may divert focus from the patient's primary reason for the visit, that is, pain. Future studies should focus on pain as the primary symptom, and the assessment should be multidimensional and multivariate.

REFERENCES

1. Bush FM, Dolwick MF. The temporomandibular joint and related orofacial disorders. Philadelphia, Pa: JB Lippincott, 1993:33.

2. Costen JB. A syndrome of ear and sinus symptoms dependent upon disturbed function of the temporomandibular joint. Ann Otol Rhinol Laryngol 1934;43:1-15.

3. Costen JB. Neuralgias and ear symptoms associated with disturbed function of the temporomandibular joint. JAMA 1936; 107:252-5.

4. Michael LA. Jaws revisited: Costen's syndrome. Ann Otol Rhinol Laryngol 1997;106:820-2.

5. Bleicker RE. Ear disturbances of temporomandibular ori-

gin. J Am Dent Assoc 1938;25:1390-9.

6. Shapiro HH, Truex RC. The temporomandibular joint and the auditory function. J Am Dent Assoc 1943;30:1147-68.

7. Sicher H. Temporomandibular articulation in mandibular overclosure. J Am Dent Assoc 1948;36:131-9.

8. Zimmermann AA. An evaluation of Costen's syndrome from an anatomic point of view. In: Sarnat BG, ed. The temporomandibular joint. Springfield, Ill: CC Thomas, 1951:82-110.

9. Hodges JM. Managing temporomandibular joint syndrome. Laryngoscope 1990;100:60-6.

10. Travell J. Referred pain from skeletal muscle. The pectoralis major syndrome of breast pain and soreness and the sternomastoid syndrome of headache and dizziness. N Y State J Med 1955;55:331-40.

11. Schwarz L. Disorders of the temporomandibular joint. Philadelphia, Pa: WB Saunders, 1959.

12. Molin C. Studies in mandibular pain dysfunction syndrome. Swed Dent J Suppl 1973;66(suppl 4):1-11.

13. Sharav Y, Tzukert A, Refaeli B. Muscle pain index in relation to pain dysfunction and dizziness associated with myofascial pain-dysfunction syndrome. Oral Surg Oral Med Oral Pathol 1978;46:742-7.

14. Gelb H, Tarte J. A two-year clinical dental evaluation of 200 cases of chronic headache: the craniocervical mandibular syndrome. J Am Dent Assoc 1975;91:1230-6.

15. Braaf MM, Rosner S. Trauma of the cervical spine as a cause of headache. J Trauma 1975;15:441-6.

16. Terrahe K. Das Zervikokraniale Syndrome in der Praxis des HNO-arztes. Laryngol Rhinol Otol (Stuttg) 1985;64:292-9.

17. Pearce JMS. Whiplash injury — a reappraisal. J Neurol Neurosurg Psychiatry 1989;52:1329-31.

18. Kopp S, Plato G, Bumann A. Die Bedeutung der oberen Kopfgelenke bei der Aeiologie von Schmerzen im Kopf-, Hals-, Nakenbereich. Dtsch Zahnarztl Z 1989;44:966-7.

19. Paszter E. Decompression of vertebral artery in cases of cervical spondylosis. Surg Neurol 1986;9:371-7.

20. Yates DAH, Smith MA. Orthopaedic pain after trauma. In: Wall PD, Melzack R, eds. Textbook of pain. Edinburgh, Scotland: Churchill Livingstone, 1994:409-21.

21. Brookes GB, Maw AR, Coleman MJ. "Costen's syndrome" — correlation or coincidence: a review of 45 patients with temporomandibular joint dysfunction, otalgia and other aural symptoms. Clin Otolaryngol 1980;5:23-36.

22. Pfaffenrath V, Dankekar R, Pollman W. Cervicogenic headache: the clinical picture, radiological findings and hypotheses on its pathophysiology. Headache 1987;27:495-9.

23. Meniere P. Sur une forme de surdite grave dependant d'une lesion de l'oreille interne. Gaz Med Paris 1861;16:29.

24. Feldman H. Die Geburt einer Krankheit, Dargestellt am Beispiel des morbus Meniere. Laryngorhinootologie 1993;72:1-8.

25. Tanioka H, Terahara A, Furuta A, et al. MR imaging of the inner ear; findings of Meniere's syndrome. Rinsho Hoshasen 1989;34:1365-70.

26. Merchant SN, Rauch SD, Nadol JB. Meniere's disease. Eur Arch Otorhinolaryngol 1995;252:63-75.

27. Hallam RS, Stephens SDG. Vestibular disorder and emotional distress. J Psychosom Res 1985;29:407-13.

28. Simpson RB, Nedzelski JM, Barber HO, Thomas MR. Psychiatric diagnosis in patients with psychogenic dizziness or severe tinnitus. J Otolaryngol 1988;17:325-30.

29. Sullivan MD, Katon W, Dobie R, Sakai C, Russu J, Harrop-Griffiths J-H. Disabling tinnitus: association with affective disorder. Gen Hosp Psychiatry 1988;10:285-91.

30. Minnigerode B, Harbrecht M. Oto-rhino-laryngologische manifestgationen Larvierter mono-oder obligosympatomatic depressions. HNO 1988;36:383-5.

31. Koenig HG, Cohen HJ, Blazer DG, Krishnan KRR, Sibert TE. Profile of depressive symptoms in younger and older medical inpatients with major depression. J Am Geriatr Soc 1993;41:1169-76.

32. Rasmussen P. Facial pain. IV. A prospective study of 1052 patients with a view of: precipitating factors, associated symptoms, objective psychiatric and neurological symptoms. Acta Neurochir (Wien) 1991;108:100-9.

33. Bush F, Whitehill JM, Martelli MF. Pain assessment in temporomandibular disorders. J Craniomandib Pract 1989;7:137-43.

34. Bush FM, Harkins SW. Pain-related limitation in activities of daily living in patients with chronic orofacial pain: psychometric properties of a disability index. J Orofacial Pain 1995;9:57-63.

35. Harkins SW, Lagua BT, Price DD, Small RE. Geriatric pain. In: Roy R, ed. Chronic pain in old age. Toronto, Canada: University of Toronto Press, 1994:155-9.

36. Melzack R. The McGill Pain Questionnaire: major properties and scoring methods. Pain 1975;1:277-99.

37. Pilowsky I, Spence ND. Illness behavior syndromes associated with intractable pain. Pain 1976;2:61-71.

38. Carson RC. Interpretative manual to the MMPI. In: Butcher JN, ed. MMPI: research developments and clinical applications. New York, NY: McGraw-Hill, 1969:279-96.

39. Cattell RB. Factor analysis: I. An introduction to essentials. II. The role of factor analysis in research. Biometrics 1965;21:405-35.

40. Muthen BO. Dichotomous factor analysis of symptom data. Sociol Methods Res 1989;18:19-65.

41. Harkins SW, Bush FM, Price DD, Hamer RM. Symptom report in orofacial pain patients: relation to chronic pain, experimental pain, illness behavior and personality. Clin J Pain 1991;7:102-13.

42. Goodfriend DJ. Deafness, tinnitus, vertigo and neuralgia. Arch Otolaryngol 1947;46:1-35.

43. Hankey GT. Some observations on Costen's mandibular syndrome: including a review of 100 out of 500 cases. Proc R Soc Med 1957;51:225-32.

44. Gelb H, Arnold GE. Syndromes of the head and neck of dental origin. I. Pain caused by mandibular dysfunction. Arch Otolaryngol 1959;70:681-91.

45. Capps FCW. Temporomandibular joint syndrome of Costen. Proc R Soc Med 1962;55:792-3.

46. Hankey GT. Painful disorders of the temporomandibular joint. Proc R Soc Med 1962;55:781-92.

47. Dolowitz DA, Ward JW, Fingerle CO, Smith CC. The role of muscular incoordination in the pathogenesis of the temporomandibular joint syndrome. Laryngoscope 1964;74:790-

801.

48. Myrhaug H. The incidence of ear symptoms in cases of malocclusion and temporomandibular joint disturbances. Br J Oral Surg 1964-65;2:28-33.

49. Vero D. Facial pain in association with temporomandibular joint dysfunction. J Laryngol Otol 1965;79:707-23.

50. Gelb H, Calderone J, Gross S, Kantor M. The role of the dentist and the otolaryngologist in evaluating temporomandibular joint syndrome. J Prosthet Dent 1967;18:497-503.

51. Bernstein JM, Mohl ND, Spiller H. Temporomandibular joint dysfunction masquerading as disease of the ear, nose and throat. Trans Am Acad Ophthalmol Otolaryngol 1969;73:1208-17.

52. Zarb GA, Thompson GW. Assessment of clinical treatment of patients with temporomandibular joint dysfunction. J Prosthet Dent 1970;24:542-54.

53. Principato JJ, Barwell DR. Biofeedback training and relaxation exercises for treatment of temporomandibular joint dysfunction. ORL 1978;86:766-8.

54. Koskinen J, Paavolainen M, Raivio M, Roschier J. Otological manifestations in temporomandibular joint dysfunction. J Oral Rehabil 1980;7:249-54.

55. Carlsson GE, Kopp S, Wedel A. Analysis of background variables in 350 patients with TMJ disorders as reported in self-administered questionnaire. Community Dent Oral Epidemiol 1982;10:47-51.

56. Gelb H, Bernstein I. Clinical evaluation of two hundred patients with temporomandibular joint syndrome. J Prosthet Dent 1983;49:234-43.

57. Thornburn DN, Blake P. Mandibular dysfunction in children with otalgia. N Z Dent J 1985;81:89-93.

58. Wedel A, Carlsson GE. Factors influencing the outcome of treatment in patients referred to a temporomandibular clinic. J Prosthet Dent 1985;54:420-6.

59. Cooper BC, Alleva A, Cooper DL, Lucente FE. Myofacial pain dysfunction: analysis of 476 patients. Laryngoscope 1986;96:1099-106.

60. Wedel A, Carlsson GE. A four year follow-up by means of a questionnaire of patients with functional disturbances of the masticatory system. J Oral Rehabil 1986;13:105-13.

61. Bush FM. Tinnitus and otalgia in temporomandibular disorders. J Prosthet Dent 1987;58:495-8.

62. Rubinstein B, Carlsson GE. Effects of stomatognathic treatment on tinnitus: a retrospective study. J Craniomandib Pract 1987;5:254-9.

63. Williamson EH. The interrelationship of internal derangements of the temporomandibular joint. Headache, vertigo, and tinnitus: a survey of 25 patients. J Craniomandib Pract 1990;8:301-6.

64. Rubinstein B, Axelsson A, Carlsson GE. Prevalence of signs and symptoms of craniomandibular disorders in tinnitus patients. J Craniomandib Disord Facial Oral Pain 1990;4:186-93.

65. Vernon J, Griest S, Press L. Attributes of tinnitus that may predict temporomandibular joint dysfunction. Cranio 1992;10:282-8.

66. Morgan DH. Tinnitus of TMJ origin: a preliminary report. J Craniomandib Pract 1992;10:124-9.

67. Chole RA, Parker WS. Tinnitus and vertigo in patients with temporomandibular disorder. Arch Otolaryngol Head Neck Surg 1992;118:817-21.

68. Cooper BC, Cooper DL. Recognizing otolaryngologic symptoms in patients with temporomandibular disorders. J Craniomandib Pract 1993;11:260-7.

69. Englund GS, Dahlqvist R, Lindelof B, et al. DMSA administration to patients with alleged mercury poisoning from dental amalgams: a placebo-controlled study. J Dent Res 1993;73:620-8.

70. Parker WS, Chole RA, Tinnitus, vertigo and temporomandibular disorders. Am J Orthod Dentofacial Orthop 1995;107:153-8.

71. Ren Y-F, Isberg A. Tinnitus in patients with temporomandibular joint internal derangement. Cranio 1995;13:75-80.

72. Gelb H, Gelb ML, Wagner ML. The relationship of tinnitus to craniocervical mandibular disorders. Cranio 1997;15:136-43.

73. Wright EF, Bifano SL. Tinnitus improvement through TMD therapy. J Am Dent Assoc 1997;128:1424-32.

74. Ciancaglini R, Loreti P, Radaelli G. Ear, nose and throat symptoms in patients with TMD: the association of symptoms according to severity of arthropathy. J Orofacial Pain 1994;8:293-7.

75. Speculand B, Goss AN, Hughes A, Spence ND, Pilowsky I. Temporo-mandibular joint dysfunction: pain and illness behavior. Pain 1983;17:139-50.

76. Harkins SW, Price DD, Braith JU. Effects of extraversion and neuroticism on experimental pain, clinical pain, and illness behavior. Pain 1989;36:209-18.

77. Bush FM, Harkins SW, Price DD. Subjective signs of temporomandibular (TM) dysfunction: chronic pain, experimental pain and pain behavior [Abstract]. Pain 1990(suppl 5):S19.

78. Suvinen T, Sunden B, Reade PC, Gerschman J. Coping strategies and illness behaviour in temporomandibular joint pain-dysfunction syndrome (TMJPDS) [Abstract]. Pain 1990 (suppl 5):S19.

79. Olson RE, Swartz RA. Depression in patients with myofascial pain-dysfunction syndrome [Abstract]. J Dent Res 1977 (suppl B56):B160.

80. Eversole LR, Stone CE, Matheson D, Kaplan H. Psychometric profiles of facial pain. Oral Surg Oral Med Oral Pathol 1985;60:269-74.

81. McCreary CP, Clark GT, Merrill RL, Flack V, Oakley ME. Psychological distress and diagnostic subgroups of temporomandibular joint patients. Pain 1991;44:29-34.

82. Waxman HM, McCreary G, Weinrit RM, Carner EA. A comparison of somatic complaints among depressed and non-depressed older persons. Gerontologist 1985;25:501-7.

83. Tyler R, Baker LJ. Difficulties experienced by tinnitus sufferers. J Speech Hear Disord 1983;48:150-4.

84. Maloney G, Matignon E, Mehta N, Forgione A. SCL-90 scores and symptoms of tinnitus-TMD, TMD patients and controls [Abstract]. J Dent Res 1995;74:222.

85. Rubinstein B. Tinnitus and craniomandibular disorders — is there a link? Swed Dent J Suppl 1993;95:1-46.

86. Harrop-Griffiths J, Katon W, Dobie R, Sakai C, Russo J. Chronic tinnitus: association with psychiatric diagnoses. J Psy-

chosom Res 1987;31:613-21.

87. Halford JB, Anderson SD. Anxiety and depression in tinnitus sufferers. J Psychosom Res 1991;35:383-90.

88. Kirsch CA, Blanchard EB, Parnes SM. Psychological characteristics of individuals high and low in their ability to cope with tinnitus. Psychosom Med 1989;51:209-17.

89. Ogutcen-Toller M, Juniper RP. Audiological evaluation of the aural symptoms in temporomandibular joint dysfunction. J Craniomaxillofac Surg 1993;21:2-8.

90. Lockwood AH, Salvi RJ, Coad BA, Wowsley MA, Wack DS, Murphy BW. The functional neuroanatomy of tinnitus. Neurology 1998;50:114-20.

91. Granick MS, Martuza RL, Parker SW, Ojemann RG, Montgomery WW. Cerebellopontine angle meningiomas: clinical manifestations and diagnosis. Ann Otol Rhinol Laryngol 1985;94:34-8.

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PHONOSURGERY

The European Laryngological Society's Workshop 1999: Phonosurgery will be held, in English, October 9-10, 1999, in Graz, Austria. For more information, contact Prof Dr G. Friedrich, Department of ENT, University of Graz, Medical School, Auenbruggerplatz 20, Graz, Austria; telephone ++43/316/385-2579; fax ++43/316/385-3549; e-mail gerhard.friedrich@kfunigraz.ac.at.

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PERIOCULAR REGION AND THE EYE: WHAT'S IMPORTANT FOR THE GENERAL OPHTHALMOLOGIST

Periocular Region and the Eye: What's Important for the General Ophthalmologist will be held in Toronto, Canada, December 3-4, 1999. For information, contact Continuing Education, Faculty of Medicine, University of Toronto, 150 College St, Room 121, Toronto, Ontario M5S 3E2, Canada; telephone (416) 978-2719; fax (416) 971-2200; e-mail audrea.martin@utoronto.ca; web site www.cme. utoronto.ca.

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OTOENDOSCOPY FOR IMPROVED PEDIATRIC CHOLESTEATOMA REMOVAL

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Our objective was to determine the usefulness of intraoperative rigid endoscopy in detecting incompletely removed cholesteatomas, and to learn whether "second-look" procedures are still needed in children. We used 30°, 2.7-mm endoscopes to evaluate the middle ears of 14 children (29 procedures) with cholesteatomas once all visible disease had been removed under the operating microscope. If residual cholesteatoma was seen, removal continued until all disease visualized with the endoscope was removed. If the cholesteatoma was not removed intact, planned exploratory surgery was performed. The rigid endoscope detected incompletely removed cholesteatomas at surgery in 7 of the 29 cases (24%). In 2 of the 11 cases (18%) judged free of cholesteatoma by both otomicroscopy and otoendoscopy, residual disease was found at planned exploratory procedures. While otoendoscopy is clearly useful in detecting incompletely removed cholesteatoma, a substantial rate of residual disease following "complete" removal suggests the continued need for planned exploratory procedures.

KEY WORDS — cholesteatoma, endoscopy, otoendoscopy.

INTRODUCTION

The application of binocular microscopy to otologic surgery revolutionized middle ear surgery. The operative microscope provides the surgeon with unmatched image clarity and depth perception, but is limited in its field of view. Even with severe angulation and the use of various middle ear mirrors, aural cholesteatoma can hide in the recesses of the middle ear such as the sinus tympani, attic, and eustachian tube orifice.[1] Canal wall–up (CWU) procedures in particular are associated with high rates of residual disease located within these areas, ranging from 10% to 43%.[2-4]

Quartz rod endoscopes hold promise as an adjunct to otomicroscopy in the surgical management of aural cholesteatomas. The rigid endoscope was advocated for middle ear surgery by Mer et al[5] in the 1960s. Since that time, technological advances have provided high-resolution telescopes with smaller diameters and angled tips allowing excellent visualization of all areas within the middle ear cleft (see Figure). This has allowed widespread application of the rigid endoscope in otologic and neurotologic procedures.[6,7] Transtympanic examination of the middle ear space can evaluate middle ear disease and can differentiate between otitis media and cholesteatoma in patients with retraction pockets.[8-10] Rigid endoscopy has been used during primary and planned, staged exploratory surgeries ("second-look" procedures) to identify and assist in removal of cholesteatoma. Several authors suggest that endoscopic surgery might replace open second-look procedures in reexamining the mastoid and middle ear space in those patients with absent or limited residual disease.[6,11-13]

The present study evaluates the ability of intraoperative rigid endoscopy to detect incompletely removed pediatric cholesteatoma within difficult-to-visualize areas of the middle ear. Further, the presence or absence of disease at the time of staged exploratory surgery was evaluated to learn whether such second-look procedures are still needed in children.

MATERIALS AND METHODS

The operative reports and office notes were reviewed for all children undergoing cholesteatoma surgery by the senior author (G.I.) in the 3-year period between September 1994 and October 1997. These were analyzed to determine the type and extent of cholesteatoma, the type of surgery performed, the presence or absence of residual disease as determined by endoscopy at the completion of cholesteatoma excision under the operating microscope, and the incidence of residual cholesteatoma at staged exploratory surgery. The site of residual disease was noted in each case.

The following protocol was used. Canal wall–down

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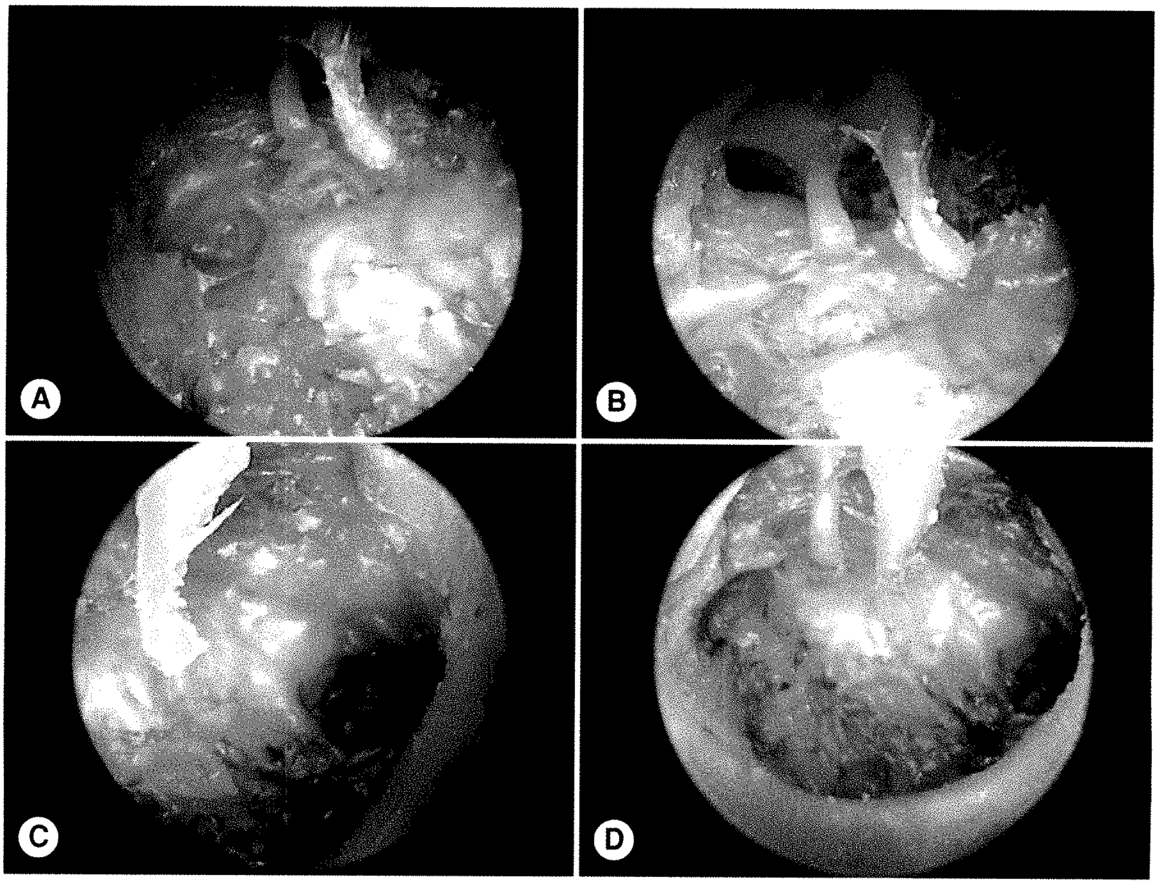

Endoscopic views of cadaver specimens. A) Sinus tympani. B) Epitympanum. C) Eustachian tube orifice. D) Hypotympanum.

(CWD) procedures were performed for extensive cholesteatomas that could not be removed without significant sacrifice of the posterior bony external auditory canal, in some patients with very contracted mastoids, and in patients in whom reliable follow-up could not be assured. When a CWU approach was used, all visible cholesteatoma was removed under the operating microscope. Next, a Storz-Hopkins 30°, 2.7-mm rigid telescope, illuminated with a 450-W xenon light source (Karl Storz Endoscopy-America, Culver City, Calif) was used to examine the middle ear, eustachian tube orifice, and aditus ad antrum by viewing directly through the optics. If cholesteatoma was noted, otomicroscopic-guided removal was accomplished with various right-angled instruments and cotton balls. Rigid endoscopy was performed periodically to assess completeness of removal. The tympanic membrane was restored in each case, usually by underlay temporalis fascia grafting.

Staged exploratory surgeries were performed if there was residual cholesteatoma that could not be removed from the middle ear, or if the cholesteatoma sac was not removed intact. These exploratory surgeries were typically scheduled 6 months later.

Photographic illustrations were obtained from cadaveric temporal bone specimens with the Storz-Hopkins 30°, 2.7-mm rigid telescope and a color video printing system.

RESULTS

Twenty-six patients ranging in age from 3 to 18 years underwent a total of 41 operations for cholesteatoma. Nine children had 10 cholesteatomas (6 acquired, 4 congenital) wherein both the initial and planned staged procedures were performed during the study period. Three children had initial CWU surgeries for cholesteatoma performed prior to the start of the study period and only planned exploratory surgeries during the study period. Two patients were referred to the senior author for additional surgery after CWU cholesteatoma surgery elsewhere. Six children underwent CWD procedures and did not undergo middle ear endoscopy. Six children (1 congenital cholesteatoma, 5 acquired) did not undergo middle ear endoscopy because of limited extent of disease (5 ears) or gross disease left on the stapes footplate (1 ear). The staged procedures totaled 19 and ranged from second- to fifth-look.

Both the operating microscope and the otoendoscope were used in 29 operations. Following presumed complete removal of the cholesteatoma matrix, 11 of the 29 operations met the criteria for planned exploratory surgery (ie, removal of cholesteatoma in more than 1 piece). The time interval between initial and staged procedures or between staged and subsequent staged procedures was 6 to 8 months (mean 6.4 months). Following the final procedure, patients were followed with periodic office otomicroscopy for 2 to 31 months (mean 12 months). None has evidence of cholesteatoma. Five patients were lost to follow-up.

In those 29 operations in which both otomicroscopy and middle ear endoscopy were performed, 7 (24%) had residual cholesteatoma discovered by endoscopy after apparent total removal under the otomicroscope. The disease identified was limited to epithelial remnants in either the sinus tympani (6 ears) or the oval window niche (1 ear). Residual cholesteatoma following apparent total removal by otomicroscopy and otoendoscopy was found in 2 of 11 (18%) planned exploratory surgeries. The pearls of cholesteatoma in these 2 cases were located in the sinus tympani (1 ear) and oval window niche (2 ears). One of these children required an additional planned exploratory surgery, which demonstrated no disease. The second has been lost to follow-up.

DISCUSSION

The optimal surgical management of aural cholesteatoma remains controversial.[14-16] Advocates of CWD surgery cite high rates of disease control with a single operation and acceptable hearing results.[17-19] Unfortunately, maintenance of the mastoid bowl that results from such surgery can be a problem in children, who often have well-pneumatized mastoids and may be uncooperative for office cleaning. Proponents of CWU surgery claim superior hearing results and improved lifestyle by avoiding a large exteriorized mastoid bowl. When such surgery is done for extensive cholesteatoma, however, there are substantial risks of both residual and recurrent disease. Several studies have demonstrated that residual disease is most common in those areas of the middle ear that are difficult to view with an operating microscope and difficult to reach with conventional instruments.[1,20] These areas include the oval window niche, sinus tympani, eustachian tube orifice, and aditus ad antrum. Thus, various adjuncts to otomicroscopy were suggested in the 1960s and 1970s to improve access to these hard-to-reach areas. The Buckingham mirror[1] and early flexible[7,8] and rigid endoscopes failed to gain widespread acceptance, as image quality was poor. Improvements in the illumination and optical quality of thin, quartz rod endoscopes have led to a reexamination of their utility in cholesteatoma surgery. Thomassin et al[20] have used rigid endoscopes for primary and revision CWU surgery for cholesteatoma since 1985 and have noted a significant decrease in the rate of residual disease, from 47% to 6%.

Our study confirms the usefulness of rigid endoscopy for cholesteatoma removal in a pediatric population. Despite meticulous dissection under the operating microscope, in 7 of the 29 cases, residual disease was found in hard-to-reach areas of the middle ear. Identification of these remnants of cholesteatoma permitted their immediate removal and eradication of disease in 5 of the 7 cases. In all of these cases, the posterior canal wall was preserved.

Among advocates of CWU surgery, neither the practice of planned exploratory surgery nor its indications are universally accepted. Planned middle ear exploration provides the opportunity to remove residual disease if present, and allows for ossicular reconstruction in a well-mucosalized middle ear with a stable tympanic membrane graft. The lysis of adhesions within the middle ear, performed during staged surgery, may also prevent retraction pocket formation. Sheehy et al[21] have reported a decline in recurrent disease from 20% to 5% following CWU procedures utilizing a staged approach in select patients. Similar results were reported by Deguine.[22] Planned exploratory surgery may lead to early detection of residual or recurrent disease and limit the number of conversions to CWD surgery. Schuring et al[4,23] were able to preserve the posterior canal wall in 85% of children requiring revision following CWU surgery by a planned staged approach.

Those who favor the single-stage approach argue that with close follow-up, residual and recurrent disease can be detected clinically before serious complications occur. Such an approach has the advantage of eliminating the morbidity of an open second-look procedure for those children who would not otherwise have recurrences. Unfortunately, Austin[18] reported a 39% recidivism rate for CWU single-stage procedures, 50% of which required a CWD procedure at the time of revision surgery. Similarly, Parisier et al[16] reported an 18% recidivism rate. Seventy-three percent of the resultant revision surgeries were CWD procedures. Neither study reported serious complications, and Parisier et al reported that the postoperative hearing was essentially the same as after the first operation.

An increasing number of otologists are individualizing their approach to cholesteatoma management, including the decision to perform a planned reexploration of the middle ear or mastoid. Identified risk factors for residual disease include young patient age, involvement of the stapes, ossicular erosion, diseased middle ear mucosa, and multiple sites of involvement.[24,25] We routinely reexplore the middle ear when cholesteatoma cannot be removed completely or when it is removed in more than 1 piece. This generally limits reexploration to cases at substantial risk for recurrence and allows for removal of microscopic residual disease. It had been our hope that routine use of middle ear endoscopy would obviate the need for routine reexplorations. This was not the case, as 2 ears thought to be free of disease by otoendoscopy and otomicroscopy had residual disease at planned exploratory surgery. In both cases, the disease was in the form of tiny, easily removed pearls.

Although this study has demonstrated that the rigid endoscope cannot reliably predict the absence of residual disease at the time of follow-up, more thorough eradication of cholesteatoma at initial surgery may limit the extent of residual disease that is present at the time of revision surgery. Shelton and Sheehy,[2] utilizing otomicroscopy alone, found that up to 85% of residual disease is in the form of a squamous pearl. Maximizing this percentage would be beneficial for several reasons. First, limited disease is often easily removed and may reduce the need for multiple surgeries. Second, some state that limited disease can be removed endoscopically, avoiding the morbidity associated with an open second-look procedure.[6,11,13]

CONCLUSION

Rigid endoscopy has become an integral part of our surgical management of pediatric cholesteatoma. In conjunction with conventional otomicroscopy, it provides improved visualization of hard-to-reach areas and the potential for more complete removal of disease. Although middle ear endoscopy use does not obviate the need for a planned exploratory surgery at this time, the rate of residual disease should significantly decrease as experience with this technique is gained.

REFERENCES

1. Bowdler DA, Walsh RM. Comparison of the otoendoscopic and microscopic anatomy of the middle ear in canal wall–up and canal wall–down temporal bone dissections. Clin Otolaryngol 1995;20:418-22.

2. Shelton C, Sheehy JL. Tympanoplasty: review of 400 staged cases. Laryngoscope 1990;100:679-81.

3. Austin DF. Staging in cholesteatoma surgery. J Laryngol Otol 1989;103:143-8.

4. Schuring AG, Lippy WH, Rizer FM, Schuring LT. Staging for cholesteatoma in the child, adolescent, and adult. Ann Otol Rhinol Laryngol 1990;99:256-60.

5. Mer SB, Derbyshire AJ, Brushenko A, Pontarelli DA. Fiberoptic endotoscopes for examining the middle ear. Arch Otolaryngol 1967;85:387-93.

6. Rosenberg SI, Silverstein H, Willcox TO, Gordon MA. Endoscopy in otology and neurotology. Am J Otol 1994;15:168-72.

7. Bottrill ID, Poe DS. Endoscope-assisted ear surgery. Am J Otol 1995;16:158-63.

8. Poe DS, Rebeiz EE, Pankratov MM, Shapshay SM. Transtympanic endoscopy of the middle ear. Laryngoscope 1992;102:993-6.

9. Takahashi H, Honjo I, Fujita A, Kurata K. Transtympanic endoscopic findings in patients with otitis media with effusion. Arch Otolaryngol Head Neck Surg 1990;116:1186-9.

10. Gonzalez C, Bluestone C. Visualization of a retraction pocket/cholesteatoma: indications for use of the middle ear telescope in children. Laryngoscope 1986;96:109-10.

11. McKennan KX. Endoscopic "second look" mastoidoscopy to rule out residual epitympanic/mastoid cholesteatoma. Laryngoscope 1993;103:810-4.

12. McKennan KX. Endoscopic transcutaneous mastoidoscopy for evaluation of residual epitympanic/mastoid cholesteatoma. Am J Otol 1993;14:369-72.

13. Rosenberg SI, Silverstein H, Hoffer M, Nichols M. Use of endoscopes for ear surgery in children. Arch Otolaryngol Head Neck Surg 1995;121:870-2.

14. Arriaga MA. Cholesteatoma in children. Otolaryngol Clin North Am 1994;27:573-91.

15. Sheehy JL, Shelton C. Tympanoplasty: to stage or not to stage. Otolaryngol Head Neck Surg 1991;104:399-407.

16. Parisier SC, Hanson MB, Han JC, Cohen AJ, Selkin BA. Pediatric cholesteatoma: an individualized, single-stage approach. Otolaryngol Head Neck Surg 1996;115:107-14.

17. Edelstein DR, Parisier SC, Ahuja GS, et al. Cholesteatoma in the pediatric age group. Ann Otol Rhinol Laryngol 1988;97:23-9.

18. Austin DF. Single-stage surgery for cholesteatoma: an actuarial analysis. Am J Otol 1989;10:419-25.

19. Hirsch BE, Kamerer DB, Doshi S. Single-stage management of cholesteatoma. Otolaryngol Head Neck Surg 1992;106:351-4.

20. Thomassin JM, Korchia D, Doris JMD. Endoscopic-guided otosurgery in the prevention of residual cholesteatomas. Laryngoscope 1993;103:939-43.

21. Sheehy JL, Brackmann DE, Graham MD. Cholesteatoma surgery: residual and recurrent disease. A review of 1,024 cases. Ann Otol Rhinol Laryngol 1977;86:451-62.

22. Deguine C. Staging in cholesteatoma surgery. Ear Nose Throat J 1993;72:197-200.

23. Schuring AG. Validating the excision of cholesteatoma. Otolaryngol Clin North Am 1989;22:1041-53.

24. Rosenfeld RM, Moura RL, Bluestone CD. Predictors of residual-recurrent cholesteatoma in children. Arch Otolaryngol Head Neck Surg 1992;118:384-91.

25. Gristwood RE, Venables WN. Factors influencing the probability of residual cholesteatoma. Ann Otol Rhinol Laryngol 1990;99:120-3.

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MANAGEMENT OF ABDUCENS PALSY IN PATIENTS WITH PETROSITIS

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Our objective was to discuss the management and outcome of abducens nerve palsy in patients with Gradenigo's syndrome. In a retrospective analysis of patients with Gradenigo's syndrome at a tertiary-care center in Houston, Texas, from 1987 to 1995, we identified 2 patients with Gradenigo's syndrome, both female. One had bilateral involvement, so that the total was 3 ears. Both patients had complete recovery of their abducens nerve palsy. In 2 ears with chronic mastoiditis, sixth nerve palsies failed to respond to medical therapy alone, but resolved after mastoidectomy with drainage of the petrous apex. We recommend that patients with Gradenigo's syndrome and evidence of chronic mastoiditis be treated with aggressive medical and surgical care.

KEY WORDS — abducens nerve palsy, Gradenigo's syndrome, mastoiditis, petrositis, petrous apicitis.

INTRODUCTION

Prior to the widespread use of antibiotics, petrous apicitis was common and often fatal. The routine use of antibiotics for otitis media has caused the incidence of acute and chronic petrous apicitis to decrease sharply.[1] However, when it does occur and if left unrecognized, it may lead to grave consequences. Complications of petrous apicitis include cranial nerve palsies, meningitis, labyrinthitis, intracranial abscess formation, retropharyngeal abscess, venous sinus thrombosis, and death.

The most common clinical findings seen in petrous apicitis are purulent otorrhea, retrobulbar and/or facial pain, sixth nerve and seventh nerve palsies, and vertigo.[2] Retrobulbar and/or facial pain in the presence of purulent otorrhea should always alert the physician to the possibility of impending petrous apicitis. When these findings are present, proper radiographic studies should be undertaken to confirm or exclude the diagnosis. Gradenigo described the classic findings of purulent otitis, trigeminal pain, and abducens nerve palsy nearly 90 years ago in patients who had purulent disease of the petrous apex. However, according to Chole and Donald,[3] only 24 of the 57 (42%) patients Gradenigo studied with this disease had this complete triad of symptoms. Although abducens palsy is not always present in patients with petrositis, it is not an uncommon finding.

Sixth nerve palsy associated with petrous apicitis is thought to be due to inflammation of the abducens nerve as it courses through Dorello's canal under the preclinoid ligament.[3] The inflammation responsible for this may be due to either acute or chronic disease within the petrous apex. Abducens palsy carries significant morbidity to the patient, yet little is written concerning its treatment and outcome in patients with petrous apicitis. This is somewhat surprising when compared to the enormous amount of literature that exists on the management of facial nerve palsy associated with suppurative mastoid disease. This may be due to the scarcity of this disease.

The purpose of this paper is to discuss the management and outcome of sixth nerve palsies in 2 patients (3 ears) with Gradenigo's syndrome. One patient was unique in that she had the syndrome bilaterally.

PATIENTS AND RESULTS

Patient 1. A 47-year-old woman presented with a 7-day history of left ear pain after an upper respiratory tract infection (URI). Two days prior to admission, she began complaining of headaches, nuchal rigidity, and fevers to 39°C. The following day, she also began experiencing left otorrhea and diplopia. The first onset of otorrhea partially relieved her ear pain. She presented to the emergency center after she began having an altered mental status. In the emergency room she had a dull intact left tympanic membrane with slight injection, an erythematous medial external auditory canal, and a small amount of purulence in the medial external auditory canal. Neurologic examination was significant for a left sixth nerve palsy, nuchal rigidity, positive Kernig's and Brudzinski's signs, and an altered sensorium. There were no other significant physical examination findings. A spinal puncture was performed and demonstrated an elevated white blood cell (WBC) count in the cerebrospinal fluid (CSF), elevated levels of protein, and

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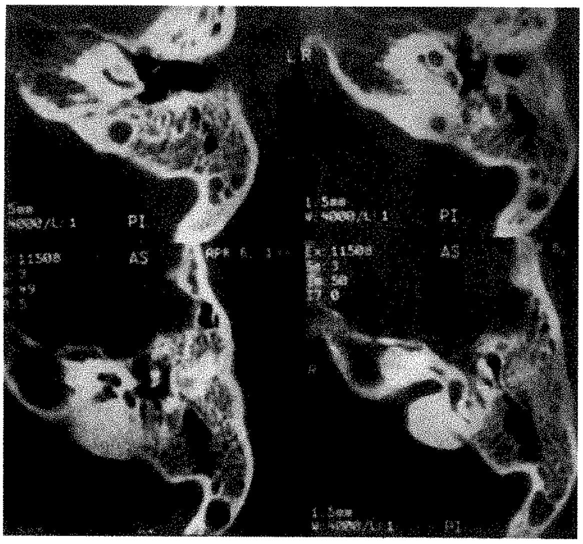

Fig 1. (Patient 1) High-resolution computed tomography of right temporal bone. Mastoid air cells are full of fluid, but there is no bony dehiscence or destruction of trabeculae.

low levels of glucose. No organisms were identified within the CSF, but latex agglutinins were positive for *Streptococcus pneumoniae*, and a diagnosis of bacterial meningitis was made. A left myringotomy was performed and a large-bore tympanostomy tube was placed. Cultures of the middle ear were inadvertently not performed. A high-resolution computed tomography (CT) scan of the temporal bones demonstrated a well-pneumatized right mastoid and a left opacified mastoid without evidence of bony erosion or coalescence (Fig 1). The patient was placed on intravenous (IV) antibiotics and her symptoms of meningismus and fevers resolved within 48 hours. Conservative management with IV antibiotics without further surgical intervention was continued. After 4 days of antibiotic therapy, her left sixth nerve palsy and symptoms of diplopia completely resolved. Oral antibiotic therapy was then continued for a total of 3 weeks. The patient was seen in the outpatient clinic 2 weeks and then 3 months after her hospital discharge and was asymptomatic.

Patient 2. A 36-year-old woman with a several-year history of bilateral chronic otitis media and a 2-day history of left ear pain, otorrhea, high-grade fevers, and a feeling of a stiff neck presented to a local hospital emergency center after being found by her children on the floor posturing and unresponsive. Two weeks prior to this incident she had been seen by her primary care physician, who placed her on cephalexin for bilateral otitis media. In the emergency center she was intubated because of decreased oxygen saturation and decorticate posturing. On examination she was disoriented and had bilateral white mucopurulent otorrhea with dull thickened tympanic membranes, bilateral sixth nerve palsies, and a House grade IV left facial paresis.

A CT scan of the brain demonstrated no abnormalities other than bilateral fluid-filled mastoid cavities. A lumbar puncture was performed and showed cloudy CSF, a WBC count of 5,625/dL, a protein concentration of more than 55 mg/dL, gram-positive cocci in pairs, and a differential of 98% neutrophils and 2% lymphocytes. The patient was transferred to Hermann Hospital and was placed on IV antibiotics (ceftriaxone sodium and vancomycin hydrochloride), dexamethasone, mannitol, and Dilantin.

Bilateral myringotomies were done and the discharge was sent for culture and sensitivity testing. After the third hospital day, the patient began follow-

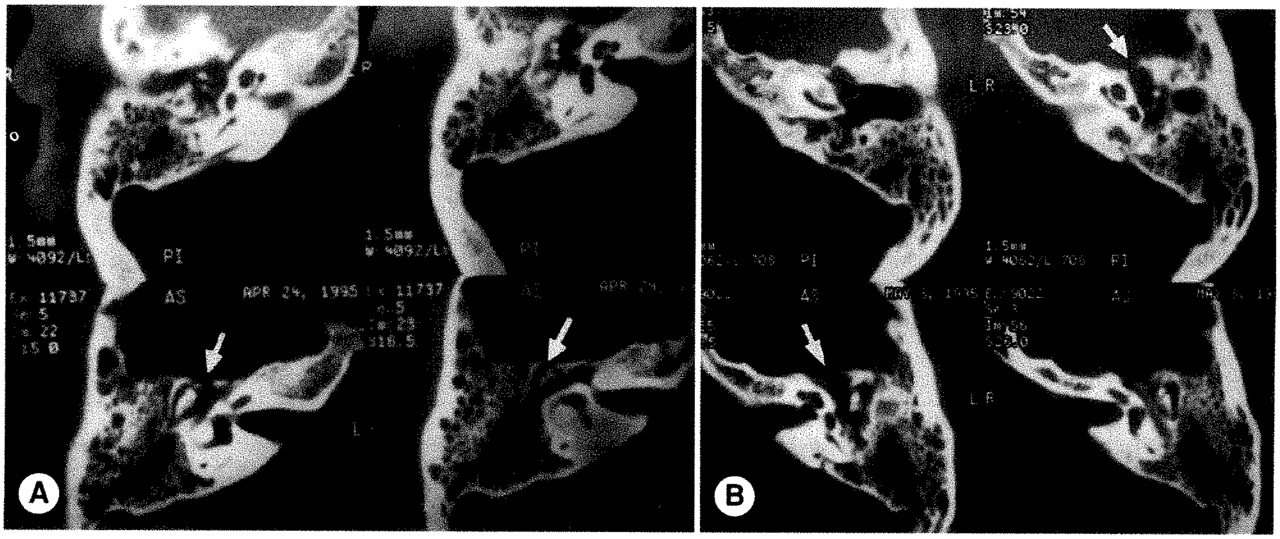

Fig 2. (Patient 2) High-resolution computed tomography of temporal bones. A) Right side. Note tegmen dehiscence (arrows) and destruction of bony trabeculae. B) Left side. Note tegmen dehiscence (arrows) and destruction of bony trabeculae causing coalescence of air cells.

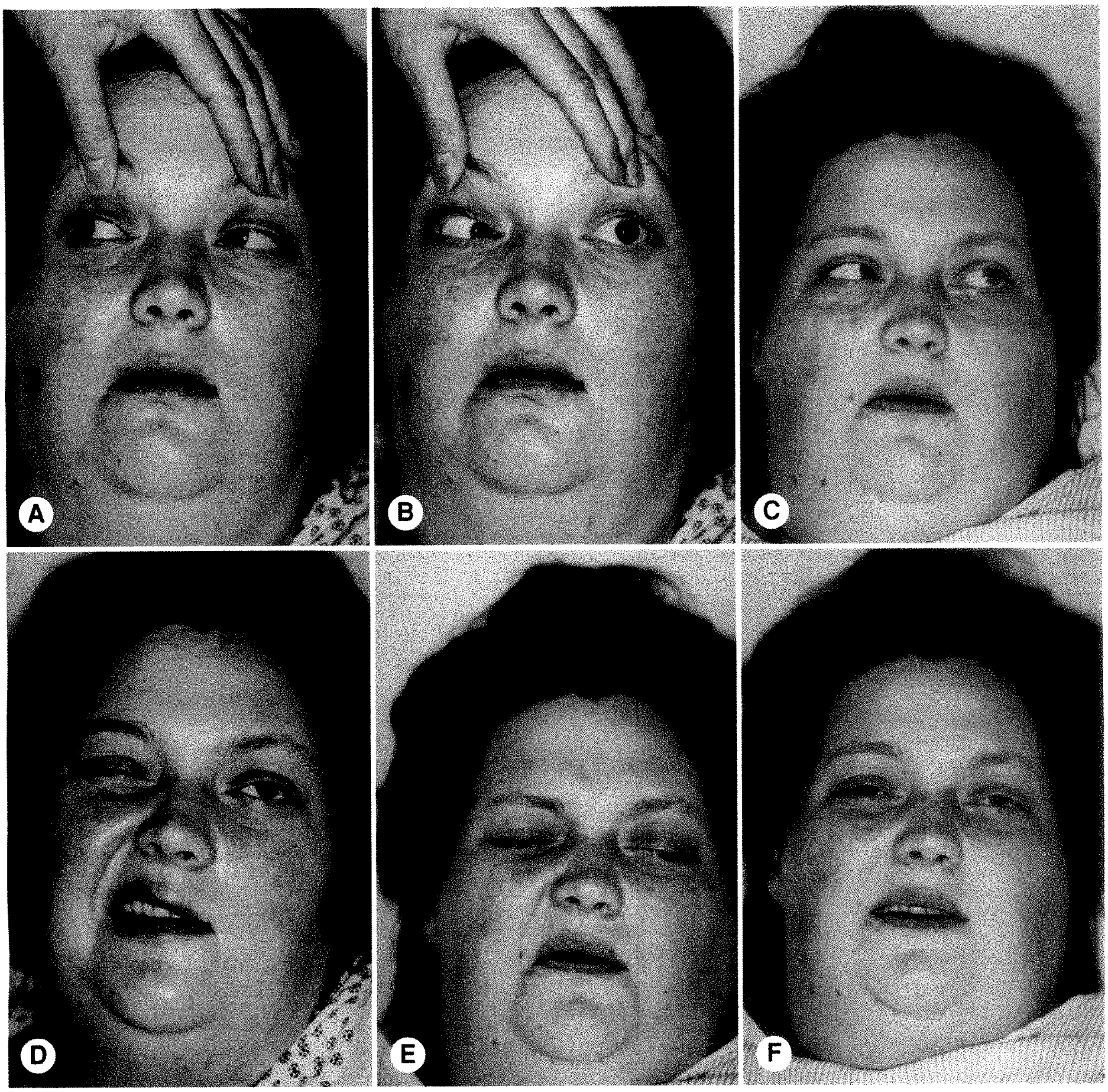

Fig 3. (Patient 2) A) Complete resolution of right abducens palsy 10 days following mastoidectomy with petrous apicectomy. B) Ten days after intravenous antibiotic therapy and prior to left mastoidectomy and petrous apicectomy. Left abducens palsy still persists. C) Five days after left mastoidectomy and petrous apicectomy. Note marked improvement in left abducens palsy following surgery. D) Demonstration of preoperative left facial palsy. E,F) Near-complete recovery of left facial palsy 7 days postoperatively.

ing commands and was extubated. At this time she was found to have a mild to moderate right hemiparesis. Magnetic resonance imaging demonstrated a small left parietal subdural empyema. Bilateral opacification of mastoid air cells with obvious bony erosion of the tegmen on the right and left sides was seen on high-resolution CT of the temporal bone (Fig 2). The CSF and otorrhea cultures grew *S pneumoniae*, and the patient was continued on IV antibiotics. Clinically, her meningitis resolved after 1 week of IV antibiotics, but she continued to have diplopia, bilateral abducens nerve palsy, and a left facial paralysis. The neurosurgical service elected to manage her small subdural empyema conservatively. On her seventh hospital day, while a neurosurgical management debate was taking place, a right mastoidectomy, petrous apicectomy, and closure of the tegmen defect with a temporalis muscle flap was performed. It was felt that a short delay prior to left mastoid surgery would not be harmful, since the patient was covered adequately by antibiotics. By the fifth postoperative day, her right sixth nerve palsy recovered (Fig 3A). Her left sixth and seventh nerve palsies, however, continued to persist despite 12 days of aggressive medical therapy (Fig 3B,D). She then underwent a left mastoidectomy, petrous apicectomy, and closure of the tegmen defect with a temporalis muscle flap. By the fifth postoperative day, following surgery on the left side, she demonstrated moderate recovery of both sixth and seventh nerve palsies (Fig 3C-F). Follow-up magnetic resonance imaging of her brain demonstrated near-complete resolution of her subdural abscess. At this time her right hemiplegia had resolved as well. Retrospectively, we see that the patient could have tolerated bilateral mastoid surgery done at the same time. She was discharged to home on the 18th hospital day in stable condition. She continued with IV antibiotics as an outpatient. On follow-up examination in the otolaryngology clinic 1 week later, she had complete recovery of all of her cranial nerve palsies.

DISCUSSION

Petrositis may be acute or chronic, depending upon the clinical course and severity of the disease. There is no doubt that pneumatization plays an important role in the spread of disease throughout the mastoid and petrous bone, but infection and inflammation may also spread to areas of nonpneumatized petrous bone by way of direct extension or venous channels.[4] Pathological changes seen in patients who have had petrositis include acute inflammation, chronic inflammation, necrosis, and fibrosis within the mucosa, bone marrow, and bone.

Acute petrositis is characterized as a nonlocalized infection with inflammatory changes involving the mucosa and bone that parallel findings in the middle ear and mastoid, whereas chronic petrositis is characteristically patterned after the histopathologic changes seen in chronic middle ear disease. Chronic inflammatory changes may be more prominent and localized in certain areas of the temporal bone, producing soft tissue that is capable of bone resorption. Similar bone resorption and inflammatory changes are also seen in patients with cholesteatoma disease.[5]

Chronic infections produce permanent thickening within the mucosal membranes of the petrous bone as a result of fibrous proliferation. This often results in a central cystic space surrounded by thickened, diseased mucosa that is surrounded by bone.[5] These areas may allow for trapping of mucopus and inflammation that make the disease particularly difficult to treat by medical therapy alone.

The first patient had classic signs and symptoms of Gradenigo's syndrome. A careful clinical history and radiographic studies revealed findings consistent with a diagnosis of acute petrositis. There was no evidence of mastoid coalescence or bony resorption on CT. For this reason we elected to manage the patient conservatively by myringotomy with tympanotomy tube placement and IV antibiotics. The patient responded very well with this therapy, and a prompt complete recovery of her sixth nerve palsy was observed. This treatment strategy was based on the premise that a majority of facial nerve palsies due to acute mastoiditis will recover with IV antibiotics, myringotomy for drainage of the middle ear and mastoid, and placement of a large-bore tympanotomy tube without further surgical intervention.[6] Generally speaking, adequate drainage of the middle ear should adequately drain the mastoid as well, in conditions in which no chronic mastoid disease or cholesteatoma is present. Under these conditions the aditus ad antrum should be patent, allowing for the passage of mucopus from the mastoid to the middle ear and through the tympanotomy tube. However, in cases in which conservative therapy fails or chronic disease is present, further surgical intervention is indicated to ensure adequate drainage of the mastoid and petrous bone. This is true in much the same way that facial nerve paralysis in a patient with chronic mastoiditis demands prompt surgical decompression of the nerve.[7]

The second patient is unique in that she had bilateral sixth nerve palsies, otitis media, and ear pain. This is an exceedingly rare condition that has only been reported in the world literature several times.[8,9] It is also interesting that this patient had a left facial palsy as well. She had a clear history and radiographic findings suggestive of chronic disease. Both sides failed to respond to 7 days of conservative management (myringotomy, tympanotomy tube placement, and IV antibiotics). No improvement in her right abducens palsy was evident until 5 days after she underwent mastoidectomy and petrous apicectomy. Likewise, her left-sided sixth and seventh nerve palsies failed to respond to an additional 5 more days of conservative therapy, and improvement was not seen until after left mastoidectomy and petrous apicectomy were done. Prompt return of both left sixth and seventh nerve function occurred only after further surgical intervention.

In a recent review of the English-language literature, we found only a small number of case reports of patients with petrositis, abducens palsy, and mention of sixth nerve palsy outcome. Historically, the treatment of petrositis has been primarily by surgical intervention.[3,6] However, in certain situations there may be a place for a more conservative approach, especially when there is no evidence of chronic ear disease. In more recent times, advances in antibiotic therapy have even led to the successful treatment of certain brain abscesses with medical therapy

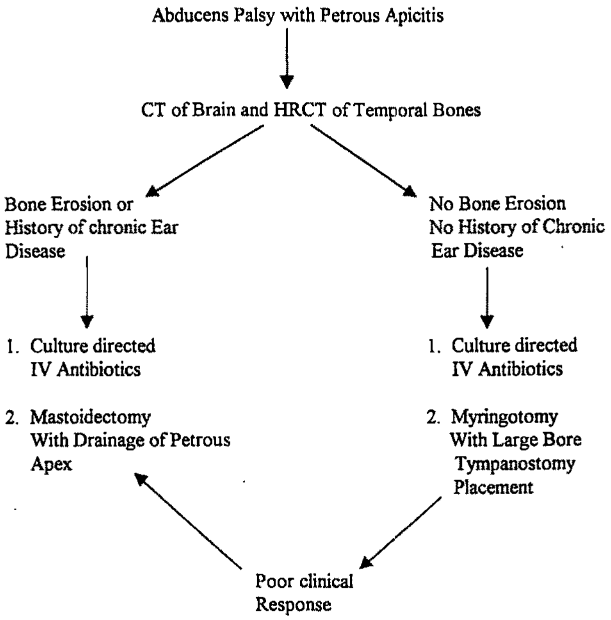
Abducens Palsy with Petrous Apicitis
CT of Brain and HRCT of Temporal Bones
Bone Erosion or History of chronic Ear Disease
No Bone Erosion No History of Chronic Ear Disease
1. Culture directed IV Antibiotics
2. Mastoidectomy With Drainage of Petrous Apex
1. Culture directed IV Antibiotics
2. Myringotomy With Large Bore Tympanostomy Placement
Poor clinical Response

Fig 4. Algorithm of management of patients with sixth nerve palsy. CT — computed tomography, HRCT — high-resolution computed tomography, IV — intravenous.

alone.[10-14]

Sternberg et al[15] followed a patient who had 3 recurrent episodes of unilateral sixth nerve palsies that occurred after a viral URI. Each time, the patient was managed conservatively and had complete return of sixth nerve function within 2 weeks.[15] Chole and Donald[3] described 1 case in a series of 8 patients with petrositis who had congenital cholesteatoma of the petrous apex, facial nerve numbness, and ipsilateral sixth nerve palsy. This occurred after a URI. Prior to his admission and diagnosis, his sixth nerve palsy and facial pain gradually resolved. This occurred over a 6-month period without surgical treatment.[3] Woody et al[16] treated a 4-year-old patient with chronic petrositis and a left abducens palsy with mastoidectomy and IV antibiotics. This patient had complete resolution of diplopia and of sixth nerve palsy within 3 weeks.[16] Hilding and Price[17] successfully treated a 9-month-old who had mastoiditis with bone destruction and a sixth nerve palsy with a complete mastoidectomy and IV antibiotics. The patient had improvement in the abducens palsy by postoperative day 11.[17] Gillanders[18] described a case of a patient with Gradenigo's syndrome, chronic mastoiditis, and osteomyelitis of the temporal bone who, after surgery and long-term antibiotics, had complete resolution of his abducens palsy within 1 month.

Both of our patients and the patients of this review all eventually had a good outcome of their sixth nerve palsies. However, whenever conservative therapy was employed in the face of chronic disease, a slower, protracted course of sixth nerve recovery occurred compared to the course in ears treated more aggressively with surgery. Patients with acute petrositis did well with prompt return of sixth nerve function on conservative therapy alone.

We recommend that all patients with complicated petrositis should have a thorough clinical history and a CT scan of the temporal bones to determine whether acute or chronic petrous disease is present. All patients with chronic disease or those who are refractory to conservative therapy alone demand aggressive surgical and medical care. Patients with acute petrositis may be treated conservatively, as outlined in Fig 4. We feel that this algorithm may be useful in assisting the clinician in choosing an optimal approach for treating complicated petrositis with sixth nerve palsy.

REFERENCES

1. Glasscock ME III. Chronic petrositis. Diagnosis and treatment. Ann Otol Rhinol Laryngol 1972;81:677-85.

2. Hendershot EL, Wood JW, Bennhoff D. The middle cranial fossa approach to the petrous apex. Laryngoscope 1976;86:658-63.

3. Chole RA, Donald PJ. Petrous apicitis. Clinical considerations. Ann Otol Rhinol Laryngol 1983;92:544-51.

4. Neely JG. Intratemporal and intracranial complications of otitis media. In: Bailey BJ, ed. Head and neck surgery–otolaryngology. Philadelphia, Pa: JB Lippincott, 1993:1607-20.

5. Allam AF, Schuknecht HF. Pathology of petrositis. Laryngoscope 1968;78:1813-32.

6. Neely JG. Complications of temporal bone infection. In: Cummings CW, ed. Otolaryngology–head and neck surgery. Vol 3. St Louis, Mo: Mosby–Year Book, 1993:2840-64.

7. Goycoolea JG. Complications of suppurative otitis media. In: Paparella MM, ed. Otolaryngology. Vol 11. Philadelphia, Pa: WB Saunders, 1991:1381-403.

8. Utrata J. Gradenigo's syndrome. Bilateral occurrence. Eye Ear Nose Throat Mon 1973;52:142-4.

9. Hehl K, Tisch M, Lampl L, Draf W, Stussak G, Frey G. Bilateral Gradenigo's syndrome. On the value of an integrated therapy concept including hyperbaric oxygenation and chronic destructive inflammation of the skull base. HNO 1992;40:318-21.

10. Spirer Z, Jurgenson V, Lazewnick R, Reider-Grossvasser I. Complete recovery from an apparent brain abscess treated without neurosurgery. Clin Pediatr (Phila) 1982;21:106-9.

11. Kamin M, Biddle D. Conservative management of focal intracranial infection. Neurology 1980;31:103-6.

12. Vaquero J, Cabezudo MJ, Leuda G. Nonsurgical resolution of a brain abscess. J Neurosurg 1980;53:726-7.

13. Berg B, Franklin G, Cuneo R, Boldrey E, Strimling B. Nonsurgical cure of brain abscess: early diagnosis and follow-up with computerized tomography. Ann Neurol 1978;3:474-8.

14. Karen G, Tyrell D. Nonsurgical treatment of brain ab-

scesses: report of two cases. Pediatr Infect Dis 1984;3:331-4.

15. Sternberg I, Ronen S, Arnon N. Recurrent, isolated, postfebrile abducens nerve palsy. J Pediatr Ophthalmol Strabismus 1980;17:323-4.

16. Woody RC, Burchett SK, Steele RW, Sullivan JA, McConnell JR. The role of computerized tomographic scan in the management of Gradenigo's syndrome: a case report. Pediatr Infect Dis 1984;3:595-7.

17. Hilding D, Price UT. Petrous apex and subarcuate fossa maturation. Laryngoscope 1987;97:1129-35.

18. Gillanders DA. Gradenigo's syndrome revisited. J Otolaryngol 1983;12:169-74.

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COEXPRESSION OF NEUROTROPHIC GROWTH FACTORS AND THEIR RECEPTORS IN HUMAN FACIAL MOTOR NEURONS

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Neuronal development and maintenance of facial motor neurons is believed to be regulated by neurotrophic growth factors. Using celloidin-embedded sections, we evaluated immunoreactivity of 11 neurotrophic factors and their receptors in facial nuclei of human brain stems (4 normal cases, and 1 from a patient with facial palsy and synkinesis). In the normal subjects, positive immunoreactivity of the growth factor neurotrophin-4 and acidic fibroblast growth factor (aFGF) was observed in facial motor neurons, as was positive immunoreactivity against ret, the receptor shared by glial cell line–derived neurotrophic factor and neurturin. Immunoreactivity was moderate for the receptor trkB and strong for trkC. In the case of partial facial palsy, surviving cells failed to show immunoreactivity against neurotrophins. However, immunoreactivity of aFGF was up-regulated in both neuronal and non-neuronal cells in this patient. Results suggest that these trophic growth factors and their receptors may protect facial neurons from secondary degeneration and promote regrowth of the facial nerve after axotomy or injury.

KEY WORDS — facial motor neurons, neurotrophic factors, receptors.

INTRODUCTION

Neurotrophic factors have complex and diverse patterns of expression and action in the central nervous system (CNS) as well as the peripheral nervous system (PNS). Various neurotrophic factors have been localized, not only in target tissue, but also within neurons themselves. They can act on both neuronal and non-neuronal cells via target-derived and non–target-derived autocrine and paracrine action.[1] A given neuronal population may respond to many different growth factors, while a single factor can act on many different neuronal populations.[2] Neuronal cells can switch their survival dependency from one trophic growth factor to another during the developmental stages.[1,2] The expression of trophic factors within any given cell type might change, depending on the specific pathological condition.[3] It is well established that neurotrophic growth factors play a critical role in the support, growth, and differentiation of neuronal cells during their development and in the survival of neurons in adults.[1] These growth factors are also important for the maintenance and regeneration of the PNS. Data suggest that certain trophic factors may provide a protective effect following nerve injury.[1-4] Experimental data support the hypothesis that trophic growth factors can promote the survival of motor neurons and accelerate neurite growth.[5,6]

Facial nerve palsy is a serious otologic disability that results from trauma, tumor removal, or viral infection.[7] Facial motor neurons located in the brain stem require neurotrophic factors for their survival. A rapid death of neurons occurs when peripheral trophic support is lost.[7] Knowledge concerning the expression of trophic growth factors and their receptors on facial motor neurons will help us understand their bioactivity and regulation in the PNS and also the pathological changes that occur with facial palsy and synkinesis. The present study was undertaken to evaluate the complex expression of trophic growth factors and their receptors in the facial motor neurons as a part of an attempt to develop a new approach to improve facial nerve regeneration by using trophic factors.

MATERIALS AND METHODS

Subjects. A total of 5 cases of celloidin-embedded human brain stem sections were obtained from the Department of Neuropathology of the University of Southern California Los Angeles County (USC-LAC) and the Departments of Neuroanatomy and Histopathology of the House Ear Institute. Four cases were from normal human subjects, with ages ranging from 7 to 46 years. One case was from a 37-year-old patient with partial bilateral facial palsy and synkinesis, resulting from surgeries for bilateral vestibular schwannomas (neurofibromatosis 2) in 1981 and

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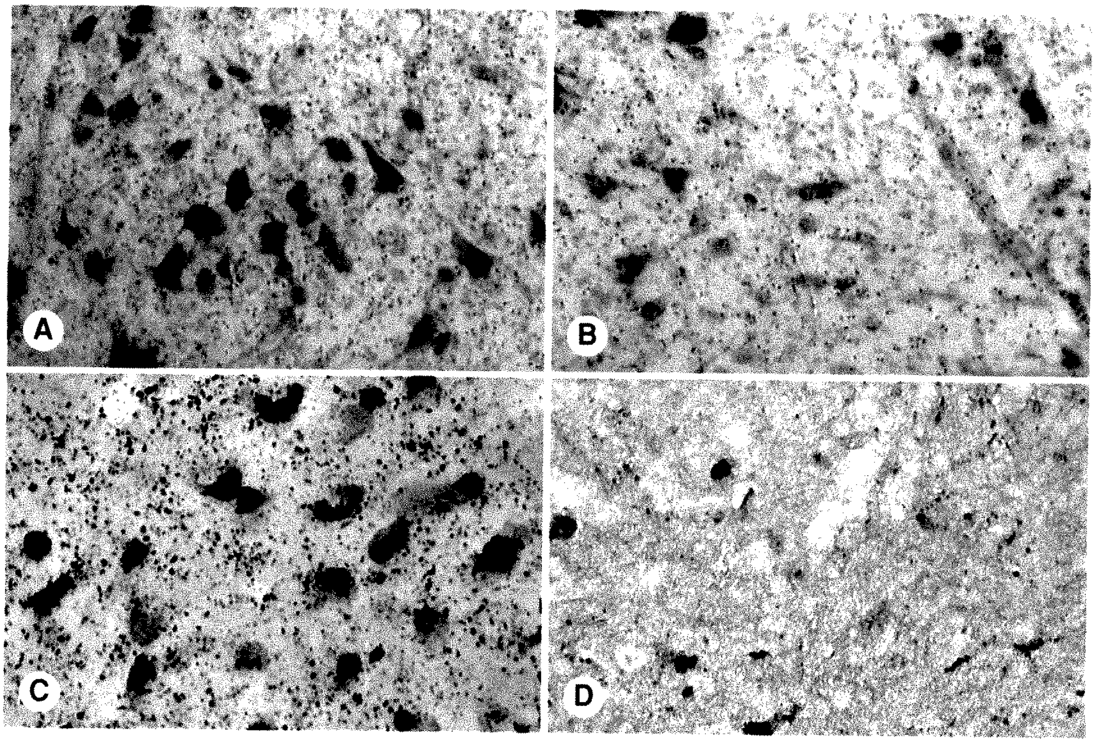

Fig 1. Original ×320. A) Nissl staining in normal human facial motor neurons. B) Nissl staining in facial nuclei in patient with partial facial paralysis and synkinesis. C) Intense trkC immunoreactivity expression in human facial neurons. D) trkC immunoreactivity in surviving cells in facial paralysis case.

1986. Immediately postoperatively, there was a House-Brackmann grade V/VI facial paralysis. Three years following the surgery, the facial nerve function had returned to a House-Brackmann grade III/VI (60% of normal) paralysis with synkinesis. There was no further improvement, and the patient died of cardiorespiratory failure in 1992.

Antibodies Used. Rabbit anti-human p75 and chick anti-human neurotrophic factor antibody package were purchased from Promega (Madison, Wis). The antibody package includes chick IgY that recognizes human brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), glial cell line–derived neurotrophic factor (GDNF), and chick anti-rat ciliary neurotrophic factor (CNTF). Rabbit anti-human trk, trkB, and trkC polyclonal antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, Calif); their IgG epitopes corresponded to amino acid 763-777, 794-808, and 812-825 maps within the highly conserved carboxyl terminus of the precursor form of trk gp140, trkB gp145, and trkC gp140. Goat anti-human ret antibody was also purchased from Santa Cruz Biotechnology. (ret is a membrane receptor mediating signaling for both GDNF and neurturin [NTN].) Its IgG epitope corresponded to amino acid 784-801 maps at the carboxyl terminus of the precursor form of the rearranged ret, which recognizes both ret proto-oncogene and rearranged ret oncogene. Rabbit anti-human acidic fibroblast growth factor (aFGF) and glial fibrillary acidic protein (GFAP) antibodies were purchased from Sigma (St Louis, Mo).

Immunohistochemistry. The method for antigen retrieval previously described was used with modifications.[8] In brief, sections were immersed in sodium hydroxide methanolate solution for 2 to 3 hours to remove celloidin, then rinsed in 2 changes in graded methanol (100%, 80%). Sections were placed in 0.3% Triton-X100 in tris-buffered saline (TBS) for 10 minutes after proteinase K digestion, then rinsed in TBS. Sections were then incubated with antibodies against individual trophic growth factors and their receptors at the following concentrations: aFGF at 1:500; GFAP at 1:80,000; others at 1:50 at room temperature overnight. Secondary antibodies (anti-chick IgY biotin conjugate, anti-rabbit and anti-goat IgG biotin conjugate) were then applied to the sections at room temperature for 2 to 4 hours. The sections were rinsed in TBS before incubation in avidin-biotin complex (ABC) at room temperature for 30 minutes to 1 hour. The chromogen solution consisted of 2.5% nickel II sulfate (optional), 0.05% 3,3-diaminobenzidine, and 0.005% hydrogen peroxide. Floating sections were loaded onto the slides and dehydrated in graded ethanol (75%, 95%, 100%), and after 3 changes of xylene, the sections were mounted with Permount and coverslipped for examination. Negative controls were stained without primary antibody or with chromogen

IMMUNOREACTIVITY OF NEUROTROPHINS AND THEIR RECEPTOR EXPRESSION IN FACIAL NUCLEI IN CELLOIDIN-EMBEDDED HUMAN BRAIN STEM SECTIONS

Antibodies	*Controls (n = 4)*	*Facial Palsy (n = 1)**
BDNF	±	−
NT-3	±	−
NT-4	++	±
CNTF	−	−
GDNF	−	−
aFGF	++	++
trk	−	−
trkB	++	−
trkC	+++	++
p75$^{\text{NTFR}}$	++	+
ret	+++	−

Tissue immunoreactivity of human brain stem sections: +++, high; ++, medium; +, low; −, not detectable; ±, unable to determine.

*Patient with facial palsy (House-Brackmann grade III/VI) and synkinesis.

solution only. The celloidin-embedded sections from the patient with partial facial paralysis were stained with Nissl's stain for histologic examination.

RESULTS

Histologic Evaluation of Facial Motor Neurons. Histologic study of the celloidin-embedded sections from the patient with partial facial palsy revealed a significant (70%) reduction of facial motor neurons relative to normal subjects (Fig 1A,B).

Immunolocalization of Neurotrophic Factors. Immunolocalization of 6 trophic growth factors — BDNF, NT-3, NT-4, CNTF, GDNF, and aFGF — was performed with various results (see Table). In normal facial motor neurons, the immunoreactivity for NT-4 and aFGF was positive (Fig 2A). It was not possible to determine BDNF or NT-3 immunoreactivity with certainty in the present study, because the immunoreactivity was too faint to distinguish from background staining. GDNF and CNTF immunoreactivities were negative (data not shown). None of these neurotrophic factors were detectable in degenerated facial motor neurons. aFGF was the only growth factor expressed in both neuronal and non-neuronal cells in the patient with facial palsy and in normal subjects (Fig 2B).

Immunolocalization of Trophic Growth Factor Receptors. Five neurotrophic growth factor receptors were identified (see Table). No trkA immunoreactivity was found in facial motor neurons. trkB, the receptor that mediates BDNF and NT-4 signaling, expressed moderate levels of immunoreactivity in normal controls (Fig 2A,D), but failed to express it in the patient with facial palsy (data not shown). trkC, the receptor responsive to NT-3, NT-4, and BDNF, exhibited intensely positive immunoreactivity in facial motor neurons (Fig 1C). Preserved trkC immunoreactivity was observed in surviving motor neu-

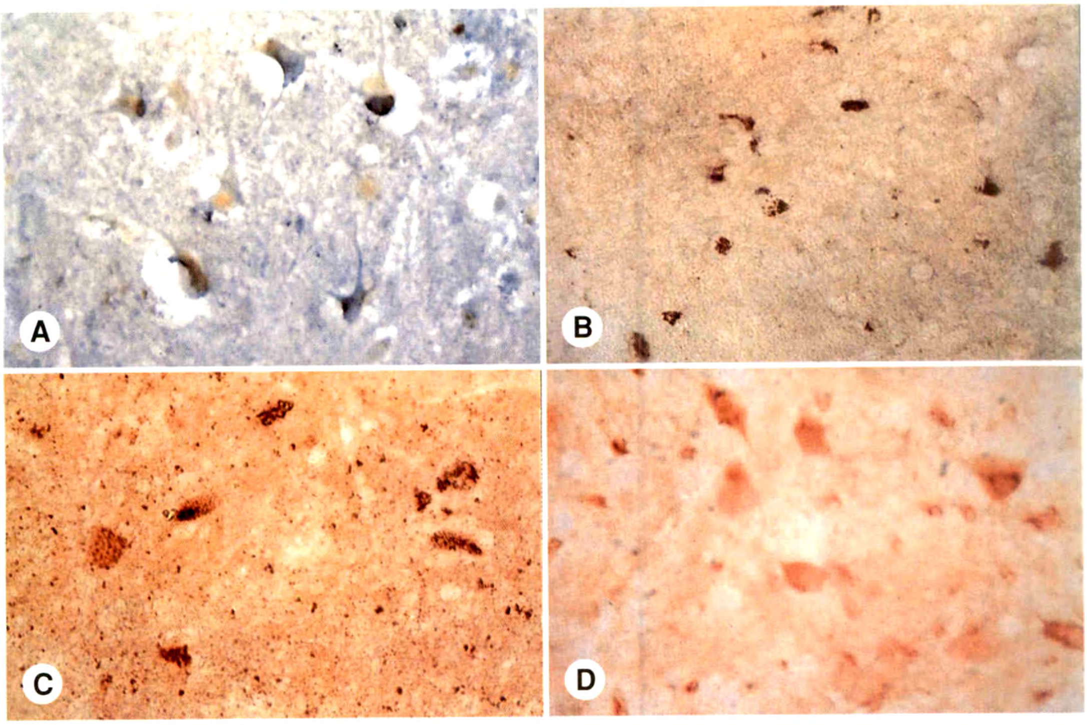

Fig 2. Neurotrophin and neurotrophic growth factor receptor immunoreactivity expression in human facial neurons (original ×320). A) Immunoreactivity of NT-4 (black) and trkB (brown) in human facial nuclei. B) aFGF immunoreactivity in brain stem in patient with facial paralysis. C) ret immunoreactivity in human facial motor neurons. D) trkB immunoreactivity in normal facial neurons.

rons in the patient with facial palsy (Fig 1C,D). p75^{NTFR}, a low-affinity nerve growth factor (NGF) receptor that has an equal affinity for each neurotrophin, expressed positive immunoreactivity in both the normal cases and the patient, but to different degrees (data not shown). ret, which mediates signaling for both GDNF and NTN, displayed strong immunoreactivity in normal facial motor neurons (Fig 2C), but was undetectable in the case of the patient with facial palsy (data not shown).

GFAP Immunoreactivity in Human Brain Stem. GFAP expressed equal immunoreactivity in glial cells in the normal human brain stems and the patient with facial palsy (data not shown). GFAP immunoreactivity was not observed in neurons, as expected.

DISCUSSION

Our attempt to establish baseline expression of neurotrophic factors and their receptors in human facial motor neurons in healthy subjects and after injury is motivated by the need to develop a rational strategy to utilize neurotrophic factors to accelerate the regeneration of the facial nerve after injury.

NT-4/trkB. In this study, NT-4 was the only detectable growth factor in the NGF family in the facial motor neurons. But its immunoreactivity was greatly decreased, to undetectable levels, in the facial palsy case. Previous studies have demonstrated that NT-4 messenger RNA (mRNA) is present in many tissues and in major regions of the brain.[9-11] In vitro, NT-4 induces differentiation of cultured neurons.[10] Although there was no BDNF immunoreactivity in facial nuclei in this study, since NT-4 and BDNF use the same trkB receptor, it is conceivable that NT-4 may provide the same biological function as BDNF.[12] Recombinant BDNF and NT-4/5 promote the survival of axotomized immature spinal motor neurons in vivo and increase the level of immunoreactivity in both choline acetyltransferase and p75^{NTFR} after nerve crush.[13,14] The results of this study suggest that NT-4 may play a major role in maintaining viability of facial motor neurons in adults in an autocrine fashion, and it may modulate the survival of motor neurons following nerve injury.

trkB is a protein tyrosine kinase that functions as the signaling receptor for both BDNF and NT-4/5. Positive trkB immunoreactivity observed in facial motor neurons in the present study is consistent with a previous study that showed that motor neurons might continue to express the full-length trkB into adulthood.[15] Coexpressed trkB and NT-4 immunoreactivity in facial motor neurons in our study may imply that in addition to their autocrine nature, BDNF and NT-4 may have a receptor-mediated paracrine action in the PNS, and play an important role in adults for survival of motor neurons after peripheral nerve injury.

NT-3/trkC. NT-3 immunoreactivity was as low as the background level in our study (data not shown). Previous studies have shown that the pattern of NT-3 mRNA expression occurs in various regions of the PNS. Its expression declines during development and reaches low levels in the adult,[16] consistent with our findings. The reasons for this down-regulation of NT-3 in mature peripheral nerves are not well understood. It is possible that the amount of NT-3 required for maintenance in the adult is not as high as that needed during the proliferative growth stages.

trkC is the receptor responsible for mediating the trophic properties of NT-3 and is widely expressed in the developing as well as adult CNS and PNS.[17] Unlike NT-3, trkC was strongly expressed in the present study. Moderate trkC immunoreactivity was also observed in surviving neuronal cells in the case with facial palsy. The results strongly indicate that this growth factor bioactivity is regulated by a receptor-mediated paracrine mechanism. We observed a significant area of overlap of immunoreactivity for trkB and trkC in motor neurons in the adult human brain stem, although their levels of expression were different. These observations together suggest that NT-4 and its cognate receptors, trkB and trkC, may cooperate with each other in facial neuron maintenance.

GDNF/ret. While GDNF immunoreactivity was negative, the immunoreactivity of its receptor ret was strongly positive in normal facial motor neurons in this study. It is possible that GDNF and ret act on facial motor neurons via a receptor-mediated paracrine action. As previously described, GDNF is synthesized as a precursor by a number of tissues and cells, including Schwann cells, and it may affect motor neurons.[18] In vitro, GDNF increases cultured motor neuron cell numbers, promotes neurite outgrowth, and increases choline acetyltransferase activity. In vivo, GDNF rescues the motor neurons from cell death following axotomy of the facial nerve in newborn rats.[19,20] GDNF can be retrogradely transported as a target-derived trophic factor for motor neurons.[19] GDNF mRNA expression is widespread in non-neuronal cells, and its alternative splicing transcription is also expressed in Schwann cells.[18] Consistent with this finding, we also observed intense GDNF immunoreactivity in human Schwann cell cultures (unpublished data). Normal ret function is necessary for GDNF signaling in the PNS.[21,22] In the facial palsy case, ret immunoreactivity was completely lost. We speculate that in the early stage of facial nerve degeneration, facial motor neurons may still preserve ret protein activity for a short time, and then it will sharply decline following the degeneration of the facial nerve. Indeed, delivery of GDNF right after axotomy or trauma rescued facial motor neurons from secondary degeneration in animals.[20] We propose that GDNF may also rescue motor neurons after axotomy in humans.

trkA and p75$^{\mathrm{NTFR}}$. Positive immunoreactivity of p75$^{\mathrm{NTFR}}$, a low-affinity receptor for the NGF family, was observed in both normal controls and the case of facial palsy at different expression levels. Previous studies showed p75$^{\mathrm{NTFR}}$ could function as a signaling molecule, and it can increase binding sites by increasing trkA expression.[23] In this study, p75$^{\mathrm{NTFR}}$ and trk (a high-affinity NGF family receptor) such as trkB and trkC may cooperate to increase response to neurotrophins during the course of facial nerve maintenance and regeneration. No positive immunoreactivity of trkA was observed in facial motor neurons.

CNTF. CNTF rescues neonatal facial motor neurons from axotomy-induced cell death in vivo.[24,25] CNTF-treated rat facial nuclei have shown up to 75% survival after axotomy, compared to untreated facial motor neurons, in which 77% to 80% died in the first 7 days.[4] CNTF immunoreactivity was not clearly demonstrated in this study. This result is consistent with a previous report showing that CNTF is not expressed in facial motor neurons themselves, but rather, is localized to myelinating Schwann cells.[26,27] Consequently, the peripheral nerve is the most abundant source of CNTF. Since we have observed CNTF immunoreactivity in human Schwann cell cultures (unpublished data), the present result may suggest a receptor-mediated paracrine action by CNTF in facial motor neurons. Although CNTF is not considered to be a target-derived factor, transport of CNTF after injury to the peripheral nerve is very robust.[28] In animal experiments, CNTF prevented the degeneration of facial motor neurons after axotomy.[4] Therefore, CNTF could be a candidate for use as a treatment in the early stage of nerve injury or axotomy in humans.

aFGF and GFAP. aFGF immunoreactivity was observed at the same level in both the normal and facial paralysis cases. The positive immunoreactivity was mostly in small neurons and supporting cells. The FGF family has 9 isoforms, and most of the members initiate fibroblast proliferation. Although it has been considered to play substantial roles in development, tissue remodeling, and prolonging neuron survival,[29] the results of this study suggest that aFGF may not predominantly influence survival of large facial motor neurons.

GFAP, a marker for glial cells, showed immunoreactivity only in glial cells, and not in neurons. The expression of GFAP in this study can be interpreted to indicate that glial cells might migrate and take the place of facial neurons after neuronal death.

SUMMARY

The survival of motor neurons and regeneration of peripheral nerves in adult animals and humans is thought to be dependent upon the availability of diffusible neurotrophic factors. These neurotrophins, however, can only exert their trophic role in those cells that express their cognate receptors. We interpret these findings to mean that multiple trophic growth factors such as NT-4, BDNF, GDNF, and aFGF could be used to protect facial neurons from secondary degeneration after axotomy and promote regrowth of the facial nerve after trauma. We hope that a new combined trophic support regimen will be developed to increase the efficiency of facial nerve regeneration.

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REFERENCES

1. Schecterson LC, Bothwell M. Novel roles for neurotrophins are suggested by BDNF and NT-3 mRNA expression in developing neurons. Neuron 1992;9:449-63.

2. Bothwell M. Functional interactions of neurotrophins and neurotrophin receptors. Annu Rev Neurosci 1995;18:223-53.

3. Lipton P, Kalil R. Neurotrophic factors — their role in development, trauma and disease. Promega Notes 1995;1:3-8.

4. Sendtner M, Kreutzberg GW, Thoenen H. Ciliary neurotrophic factor prevents the degeneration of motor neurons after axotomy. Nature 1990;345:440-1.

5. Lindsay RM, Wiegand SJ, Altar CA, DiStefano PS. Neurotrophic factors: from molecule to man. Trends Neurosci 1994; 17:182-90.

6. Henderson CE, Camu W, Mettling C, et al. Neurotrophins promote motor neuron survival and are present in embryonic limb bud. Nature 1993;363:266-70.

7. Walter MA. Facial nerve regeneration, growth factors, and return of the mimetic, symmetrical smile. Facial Plast Surg 1993;9:68-73.

8. Shi SR, Tandon AK, Haussmann RR, Kalra KI, Taylor CR. Immunohistochemical study of intermediate filament proteins on routinely processed, celloidin-embedded human temporal bone sections by using a new technique for antigen retrieval. Acta Otolaryngol (Stockh) 1993;113:48-54.

9. Hyman C, Juhasz M, Jackson C, Wright P, Ip NY, Lindsay RM. Overlapping and distinct actions of the neurotrophins

BDNF, NT-3, and NT-4/5 on cultured dopaminergic and GABAergic neurons of the ventral mesencephalon. J Neurosci 1994;14: 335-47.

10. Gao WQ, Zheng JL, Karihaloo M. Neurotrophin-4/5 (NT-4/5) and brain-derived neurotrophic factor (BDNF) act at later stage of cerebellar granule cell differentiation. J Neurosci 1995; 15:2656-67.

11. Davies AM, Horton A, Burton LE, Schmelzer C, Vandlen R, Rosenthal A. Neurotrophin-4/5 is a mammalian-specific survival factor for distinct populations of sensory neurons. J Neurosci 1993;13:4961-7.

12. Morse JK, Wiegand SJ, Anderson K, et al. Brain-derived neurotrophic factor (BDNF) prevents the degeneration of medial septal cholinergic neurons following fimbria transection. J Neurosci 1993;13:4146-56.

13. Friedman B, Kleinfeld D, Ip NY, et al. BDNF and NT-4/5 exert neurotrophic influences on injured adult spinal motor neurons. J Neurosci 1995;15:1044-56.

14. Griesbeck O, Parsadanian AS, Sendtner M, Thoenen H. Expression of neurotrophins in skeletal muscle: quantitative comparison and significance for motoneuron survival and maintenance of function. J Neurosci Res 1995;42:21-43.

15. Koliatsos VE, Clatterbuck RE, Winslow JW, Cayouette MH, Price DL. Evidence that brain-derived neurotrophic factor is a trophic factor for motor neurons in vivo. Neuron 1993;10: 359-67.

16. Maisonpierre PC, Belluscio L, Friedman B, et al. NT-3, BDNF and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression. Neuron 1990;5:501-9.

17. Lamballe F, Klein R, Barbacid M. TrkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. Cell 1991;66:967-79.

18. Henderson CE, Phillips HS, Pollock RA, et al. GDNF: a potent survival factor for motoneurons present in peripheral nerve and muscle. Science 1994;266:1062-4.

19. Yan Q, Matheson C, Lopez O. In vivo neurotrophic effects of GDNF on neonatal and adult facial motor neurons. Nature 1995;373:341-4.

20. Matheson CR, Wang J, Collins FD, Yan Q. Long-term survival effects of GDNF on neonatal rat facial motoneurons after axotomy. Neuroreport 1997;8:1739-42.

21. Rosenthal A. Multicomponent receptors for the GDNF/neurturin protein family. Neural Notes 1997;3:3-6.

22. Durbec P, Marcos-Gutierrez CV, Kilkenny C, et al. GDNF signalling through the ret receptor tyrosine kinase. Nature 1996; 381:789-93.

23. Mahadeo D, Kaplan L, Chao MV, Hempstead BL. High affinity nerve growth factor binding displays a faster rate of association than p140 trk binding. Implications for multi-subunit polypeptide receptors. J Biol Chem 1994;269:6884-91.

24. Sendtner M, Dittrich F, Hughes RA, Thoenen H. Actions of CNTF and neurotrophins on degenerating motoneurons: preclinical studies and clinical implications. J Neurol Sci 1994; 124(suppl):77-83.

25. Clatterbuck RE, Price DL, Koliatsos VE. Further characterization of the effects of brain-derived neurotrophic factor and ciliary neurotrophic factor on axotomized neonatal and adult mammalian motor neurons. J Comp Neurol 1994;342:45-56.

26. Friedman B, Scherer SS, Rudge JS, et al. Regulation of ciliary neurotrophic factor expression in myelin-related Schwann cells in vivo. Neuron 1992;9:295-305.

27. Seniuk N, Altares M, Dunn R, Richardson PM. Decreased synthesis of ciliary neurotrophic factor in degenerating peripheral nerves. Brain Res 1992;572:300-2.

28. Helgren ME, Squinto SP, Davis HI, et al. Trophic effect of ciliary neurotrophic factor on denervated skeletal muscle. Cell 1994;76:493-504.

29. Burgess WH, Maciag T. The heparin-binding (fibroblast) growth factor family of proteins. Annu Rev Biochem 1989;58: 575-606.

IMAGING CASE STUDY OF THE MONTH

FDG PET IMAGING OF MALIGNANT PARAGANGLIOMA OF THE NECK

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INTRODUCTION

Cervical paragangliomas are uncommon neoplasms derived from neural crest cells, and are also referred to as chemodectomas, carotid body tumors, glomus vagale tumors, or glomus tympanicum tumors, depending upon their structure of origin. These are considered to be benign neoplasms that grow slowly and metastasize only sporadically (12% of carotid body tumors).[1,2] However, their biologic behavior cannot be predicted by light microscopy, and the most reliable criterion of malignancy is local invasion or metastasis to lymph nodes, bone, liver, and lung. More than 100 cases of malignant paraganglioma have been reported, but the number of cases with distant metastases is small.[3] Those are usually characterized by a slow clinical course.[1] Imaging techniques rely on the high vascularity of the tumors, while scintigraphy exploits their neuroendocrine heritage with ^{123}I-metaiodobenzyl guanidine (MIBG) and somatostatin receptor agonists. 2-[^{18}F]-fluoro-2-deoxy-D-glucose positron emission computed tomography (FDG PET) has proven its high accuracy for the detection of malignancies of all kinds using glucose metabolism, independent of specific amine uptake mechanisms or cell surface receptors.[4] This is the first report demonstrating the ability of FDG PET to depict metastatic paraganglioma.

CASE REPORT

A 71-year-old woman with a history of hypertonia, cardiac arrhythmia, and mild asthma presented with otalgia, globus sensation, dysphonia, and a firm

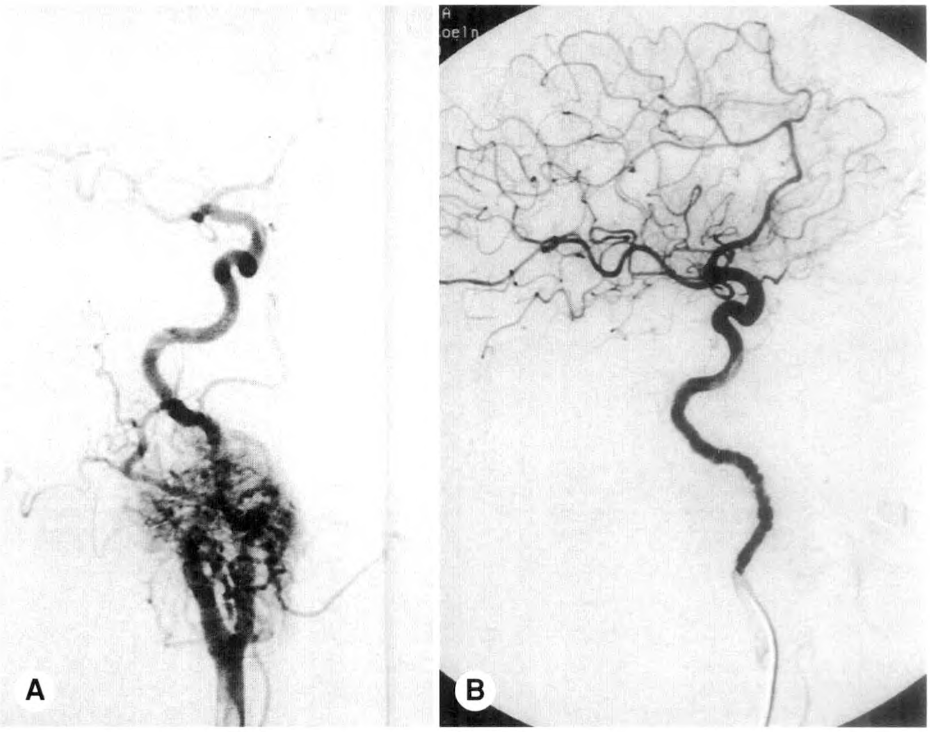

Fig 1. Digital subtraction angiography of right carotid artery, using 250 mL Visipaque 270. A) Angiogram of right common carotid artery showing large vasculated mass situated above carotid bifurcation; pathognomonic feature of carotid artery body tumor. B) Selective angiogram of right internal carotid artery; irregular lumen width and strictures are clearly depicted. Moreover, invisibility of tumor in this selective internal carotid artery angiogram proves blood supply by external carotid artery.

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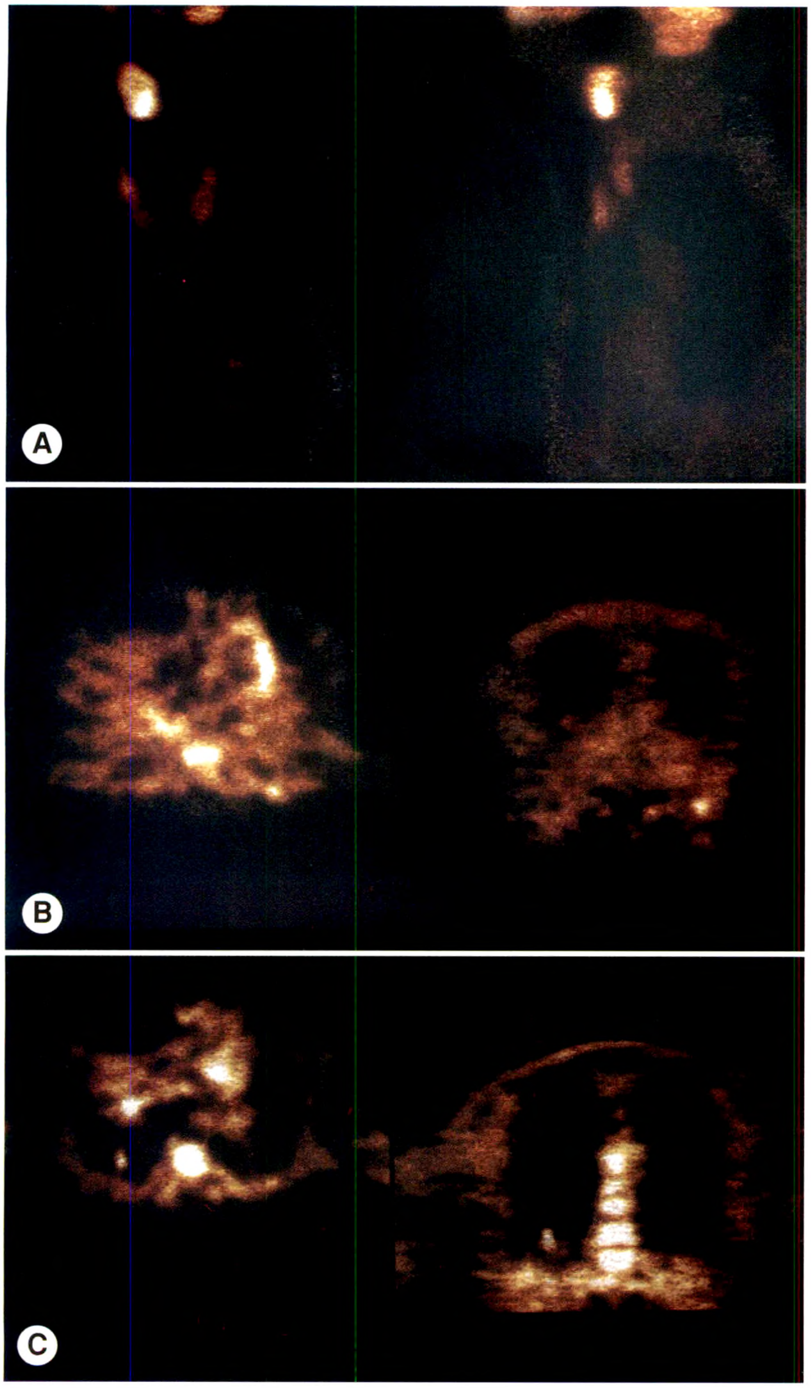


Fig 2. FDG PET fusion images in tridimensional orientation. A) Cervical paraganglioma with high ^{18}FDG uptake. B) Thoracic tridimensional FDG PET showing ^{18}FDG accumulation in left ninth lung segment. C) Accumulation of tracer in right lung segment No. 10.

right neck mass that had not resolved despite multiple courses of antibiotics. Ultrasound depicted a large lesion, in close contact with the right carotid bifurcation, with indistinct borders and inhomogeneous echo intensity. The internal carotid artery seemed to be compressed. Carotid angiography revealed a vascular lesion at the right carotid bifurcation, supplied by the right external carotid artery and the right thyrocervical trunk, with characteristic voluminous veins draining to both sides of the neck (Fig 1). The right internal carotid artery walls seemed to be contracted and narrowed by the tumor (Fig 1).

Exploration of the right side of the neck revealed a fleshy violaceous mass located between the internal jugular vein and the internal carotid artery. A partial resection of the tumor was made and the incision was closed. Histologic examination including immunohistochemical staining with neuron-specific enolase and chromagranin was consistent with a paraganglioma. However, histologic techniques were unable to evaluate the malignancy of the tumor.

A PET study was performed. After administration of 370 mBq ^{18}FDG, an ECAT exact scanner (CTT/

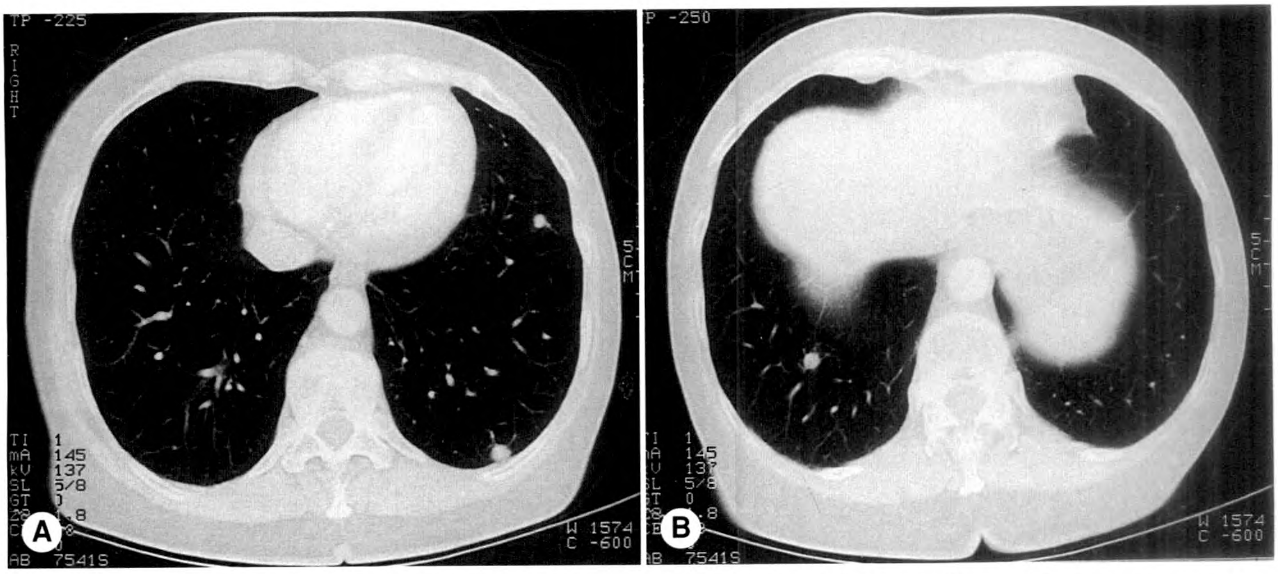


Fig 3. Spiral CT slices after water-soluble iodine contrast agent administration, depicting small lung metastases, corresponding to A) Fig 2B and B) Fig 2C.

Siemens) transmission scan was used for correction of the emission tomograms and for image fusion.[5] The PET images showed intense accumulation of FDG within the tumor and additionally revealed the presence of multiple lung metastases (Fig 2). A computed tomography (CT) scan of the lung and the abdomen confirmed multiple lesions, considered metastatic, in both lungs (Fig 3). A CT-aided biopsy of suspicious lesions in the lung was carried out unsuccessfully. The patient refused surgical exploration of the pleura and left the university clinics a few days later without further treatment. Two years later, the patient is still alive; she suffers from vocal cord palsy, dyspnea, and growing cervical masses.

DISCUSSION

Although paragangliomas are usually benign and slowly growing tumors, surgery is the mainstay of treatment, as significant symptoms will arise from local mass effects of the tumor. Additionally, early surgery may prevent intracranial extension and the development of metastases.[6,7] However, the malignant clinical course is slow in the majority of cases, and the risk of permanent cranial nerve deficit after excision of cervical paragangliomas ranges up to 40% and is the most important cause of postoperative morbidity.[1,6,7] Histologically, a distinction between benign, locally invasive, and malignant paragangliomas is usually not possible. The tumors are at present judged as malignant mainly on the basis of clinical evidence.[8]

Precise determination of tumor sites and their relation to adjacent structures is essential, since it influences the therapeutic strategy. Imaging techniques for localization of cervical paraganglioma are CT, magnetic resonance imaging (MRI), angiography, and MIBG scintigraphy.[9]

In evaluating paragangliomas before surgery, cross-sectional imaging modalities such as CT and MRI play a major role because they provide the morphological information that is vitally important to surgeons. Additionally, Maurea et al[10] reported CT and MRI to have a significantly higher diagnostic sensitivity than MIBG scintigraphy. Nevertheless, these techniques may fail to detect tumor recurrences in regions of previous surgery. Additionally, MIBG scintigraphy allows localization of distant tumor sites and metastases.

The typical site and hypervascularity of carotid body tumors has been described by contrast angiography.[11] It delineates the extent of tumor accurately, shows the tumor's blood supply, and excludes the presence of carotid artery invasion. However, it is invasive. Recent advances in interventional radiography permit relatively safe and effective embolization of cervical and skull base paragangliomas.[12] Preoperative embolization seems to reduce intraoperative blood loss and to cause a necrosis with reduction of the tumor size. Unfortunately, stroke, cranial nerve palsies, and uncontrollable fluctuations of blood pressure can occur after embolic therapy.[12]

The neural crest origin of the tumors and the presence of biogenic amine uptake and storage is exploited by MIBG scintigraphy. Van Gils et al[9] demonstrated tumor uptake of ^{123}I-MIBG in 8 of 15 patients with known chemodectomas. The sensitivity of ^{131}I-MIBG appears slightly lower, with uptake described in known lesions in 4 of 10 patients.[13] Additionally, the presence of an adjacent tumor may be masked by the normal marked salivary gland uptake of MIBG.[14] However, the ability to study the whole body in a single examination is useful for seeking distant metastases or eventually coexistent neuroendocrine tumors like pheochromocytoma. Therefore, some authors recommend the use of MIBG scintigraphy.[15]

The substantial elevation of glucose uptake and retention by tumors relative to most non-neoplastic tissues is fundamental to FDG PET imaging in oncology.[16] This approach has been successful in visualizing neuroendocrine tumors in cases in which the specific cellular mechanisms necessary for imaging with MIBG are absent.[15] Macfarlane et al[17] reported successful detection of benign cervical paraganglioma with FDG PET in 2 patients. In our patient, we could clearly demonstrate the tumor in the known site of the right neck, and we were also able to reveal multiple distant metastases in the lung and liver by using FDG PET imaging. This case suggests that FDG PET imaging may be a useful tool in the detection of both benign and malignant cervical paragangliomas.

REFERENCES

1. Vuorela AL, Jakobsson M, Anttinen J. Slowly growing pulmonary metastases of malignant cervical chemodectoma. Acta Oncol 1993;32:77-8.

2. Zbaren P, Lehmann W. Carotid body paraganglioma with metastases. Laryngoscope 1985;95:450-4.

3. Massey V, Wallner K. Treatment of metastatic chemodectoma. Cancer 1992;69:790-2.

4. Bailet JW, Abemayor E, Jabour BA, Hawkins RA, Ho C, Ward PH. Positron emission tomography: a new, precise imaging modality for the detection of primary head and neck tumors and assessment of cervical adenopathy. Laryngoscope 1992;102:281-8.

5. Pietrzyk U, Herholz K, Schuster A, von Stockhausen H, Lucht H, Heiss WD. Clinical applications of registration and fusion of multimodality brain images from PET, SPECT, CT, and MRI. Eur J Radiol 1996;21:174-82.

6. Marchesi M, Biffoni M, Nobili-Benedetti R, et al. Surgical treatment of paragangliomas of the neck. Int Surg 1997;82:394-7.

7. van der Mey AGL, Frijns JHM, Cornelisse CJ, et al. Does intervention improve the natural course of glomus tumors? A series of 108 patients seen in a 32-year period. Ann Otol Rhinol Laryngol 1992;101:635-42.

8. Lawson W. The neuroendocrinic nature of the glomus cells: an experimental, ultrastructural and histochemical tissue culture study. Laryngoscope 1980;90:120-44.

9. van Gils APG, van Erkel AR, Falke THM, Pauwels EKJ. Magnetic resonance imaging or metaiodobenzylguanidine scintigraphy for the demonstration of paragangliomas? Eur J Nucl Med 1994;21:239-53.

10. Maurea S, Cuocolo A, Reynolds JC, Neumann RD, Salvatore M. Diagnostic imaging in patients with paragangliomas. Q J Nucl Med 1996;40:365-71.

11. Brismar J. Angiography of chemodectomas of the neck. Acta Radiol 1980;21:689-96.

12. Valavanis A. Preoperative embolization of the head and neck: indications, patient selection, goals, precautions. AJNR Am J Neuroradiol 1986;7:943-52.

13. van Moll L, McEwan AJ, Shapiro B, et al. Iodine-131 MIBG scintigraphy of neuroendocrinic tumors other than pheochromocytoma and neuroblastoma. J Nucl Med 1987;28:979-88.

14. Weissmann AF, Gonzalez CE, Shapiro B, Shulkin B, Francis IR, Leach K. Multiple chemodectomas. Carotid body tumor masked by salivary gland uptake on I-123 MIBG scintigraphy. Clin Nucl Med 1994;19:527-31.

15. Shulkin BL, Koeppe RA, Francis IR, Deeb GM, Lloyd RV, Thompson NW. FDG PET localization of pheochromocytomas which fail to accumulate MIBG. Radiology 1993;186:711-5.

16. Reske SN, Bares R, Büll U, Guhlmann A, Moser E, Wannenmacher MF. Clinical value of positron emission tomography (PET) in oncologic questions: results of an interdisciplinary consensus conference. Schirmerreschaft der Deutschen Gesellschaft for Nuklearmedizin. Nuklearmedizin 1996;35:42-52.

17. Macfarlane DJ, Shulkin BL, Murphy K, Wolf GT. FDG PET imaging of paragangliomas of the neck: comparison with MIBG SPECT. J Nucl Med 1995;22:1347-50.

SOFTWARE REVIEW

ENDNOTE

Company: Niles Software, Inc, 800 Jones St, Berkeley, CA 94710; http://www.niles.com.

Hardware requirements: available for Macintosh computer (at least 770 K of free memory) and for those running Microsoft Windows 3X, Windows 95, or Windows NT (386DX/25 MHz processor — or higher — and a minimum of 4 MB of RAM).

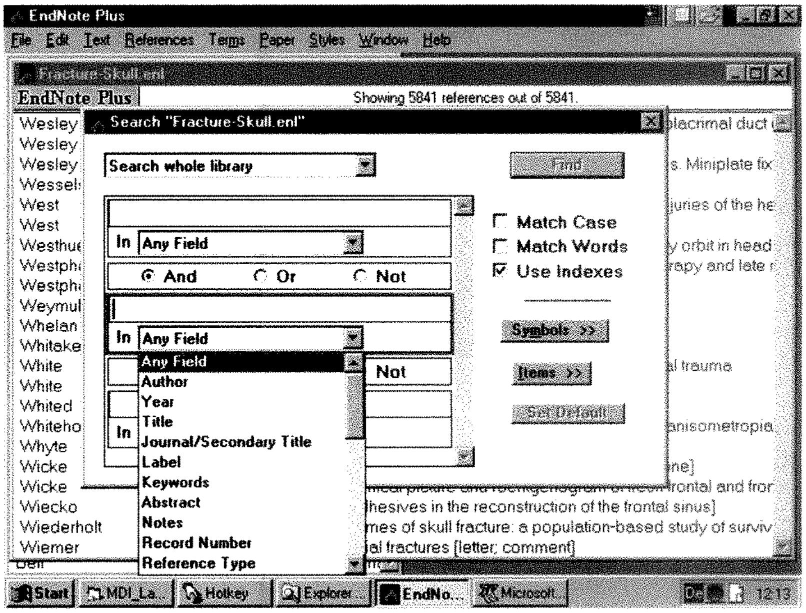
A good reference management program should make it easy to record the elements that a reference comprises, eg, author's name, year of publication, title of article, etc. It should offer tools that let one find and retrieve references quickly, and it should be able to produce the bibliography in the format required for a particular publication. There are many computer programs, but very few stand out as truly useful, time-saving, and work-enhancing. One of them is EndNote.

The functions and use of the software package EndNote for Windows are here described. With EndNote installed and running, and with a word processing file open, one can write and cite on the fly. Niles Software provides an "add-in" that is efficiently integrated into the word processor, now also for Microsoft Word 97 and WordPerfect 8.0.

Niles Software provides the ability to import references from on-line databases through a separate module called EndLink, which is integrated in EndNote in the newest version (3.0). One can create a database or download batches of references with the help of filters from one of the popular search services (eg, Medline), and the program supports major on-line or CD-ROM formats. The creation of a new filter is very easy.

The library window is intuitive and easy to navigate. The sort is by author's last name and then by date. One enters references into the databases through an edit window, tabbing from field to field as one provides the required information. There are fields for abstracts, key words, and notes. Each library can contain 32,000 references (32 megabytes), and there is no limit to the number of libraries one can have. A library can be copied and renamed at will.

This program is a breeze to use during writing. One can keep it open along with a word processing program. To cite a reference, one simply pastes the reference wherever the in-text citation should appear. EndNote inserts the author's name and the publica-



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tion number, which are used to generate the bibliography. To prepare the bibliography, EndNote scans the article, replaces the placeholders with citations, and prints the list of references at the end of the manuscript according to the style chosen. If one later resubmits the paper to another journal that requires another citation style, the program will repeat the process with the new style rules.

More sophisticated searching is also available. One can search through every word in the database. One can connect multiple search items with the Boolean operators "and," "or," and "not" (see Figure). In addition, there is a simple command for locating duplicate entries. EndNote supports multiple types of references (eg, journal, book, edited book, book section). Frequently used formatting styles such as those of *Science* or *The New England Journal of Medicine* can serve as templates to be edited. (The program will hold more than 300 journal styles.) The creation of a new style is also easy. With the new version of the program, it is now possible to generate bibliographies directly from the database for the times one wants to use a reference list in a classroom rather than in a paper. EndNote includes preformatted lists for authors, journals, and key words, and offers the capacity for up to 28 additional term lists. The journal term list can store various abbreviations of the journal name. The manual for EndNote is 418 pages and is well-written.

Altogether, EndNote provides an excellent combination of features and ease of use. It is an excellent program.

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SURFACTANT PROTEIN A IN RABBIT SINUS AND MIDDLE EAR MUCOSA

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In the present study, pulmonary surfactant protein A (SP-A) messenger RNA (mRNA) and protein were characterized in adult rabbit middle ear and maxillary sinus. Fifteen adult rabbits were used for the study: 6 with evidence of acute middle ear infections and maxillary sinusitis, 6 with infections that were successfully treated with tetracycline, and 3 that were pathogen-free. We detected SP-A mRNA in maxillary sinus and middle ear tissues by Northern blot analysis and reverse transcriptase–polymerase chain reaction (RT-PCR). The RT-PCR also revealed the presence of SP-B and SP-C mRNA in middle ear and sinus tissues. We detected SP-A protein, of molecular weight approximately 29 and 70 kd, in middle ear and sinus tissues by immunoblot analysis. Unlike the SP-A protein present in the lung, the molecular weight of the SP-A protein present in the middle ear and paranasal sinus was not altered by digestion with an enzyme that cleaves N-linked carbohydrates. Immunostaining and in situ hybridization showed that SP-A protein and mRNA, respectively, were present in surface epithelial cells of the middle ear and in epithelial cells of submucosal glands in sinus tissues. These data provide the first evidence of the presence of pulmonary surfactant proteins in the paranasal sinuses and confirm previous reports of SP-A in the middle ear epithelium.

KEY WORDS — middle ear, paranasal sinus, SP-A, SP-B, SP-C, surfactant proteins.

INTRODUCTION

Acute sinusitis and otitis media are common infectious processes in the human population.[1,2] Adequate treatment of these diseases frequently necessitates antibiotic therapy and, in chronic or recurrent cases refractory to medical therapy, may require surgical intervention.[1,2] Thus, a greater understanding of the pathophysiological processes involved in sinusitis and middle ear infections could lead to new and more effective treatment options.

It has been reported that immunoreactive pulmonary surfactant protein A (SP-A), a pulmonary surfactant–associated protein that is thought to facilitate the clearance of microbial pathogens from the lung alveolus, is present in the human middle ear.[3,4] The middle ear cavity is lined by respiratory epithelium, as are the eustachian tube, paranasal sinuses, and conducting airways of the lung.[5,6] Respiratory epithelium consists of pseudostratified, ciliated, columnar epithelium with goblet cells that overlies mixed serous-mucous submucosal glands.[6] It has been reported that in the human, SP-A is present in submucosal glands in the conducting airways of the lung.[7-9]

Pulmonary surfactant is a lipoprotein substance, synthesized and secreted by the alveolar type II cell, that reduces surface tension at the air-alveolus interface.[10] Surfactant is required for proper lung function, and a deficiency of surfactant in premature human newborns results in neonatal respiratory distress syndrome, a leading cause of neonatal morbidity and mortality.[11] Interestingly, a surfactant-like material has been identified in washings from the middle ear cavity and the eustachian tube that reduces surface tension and contains phospholipids that are characteristic of lung surfactant.[12-14] It has been proposed that the surfactant-like material present in the middle ear and eustachian tube prevents collapse of the eustachian tube during normal functioning.[12-14]

Pulmonary surfactant consists of (approximately) 80% phospholipid, 10% cholesterol, and 10% protein.[10] Surfactant protein A, the most abundant and best-characterized surfactant-associated protein, is an approximately 35 kd sialoglycoprotein.[15] It regulates surfactant homeostasis in the alveolus by influencing the synthesis and secretion of surfactant phospholipids and by regulating surfactant recycling by the alveolar type II cell.[15] It also plays a role in local defense mechanisms at the level of the alveolus.[16] For example, SP-A has been shown to activate alveolar macrophages.[17] Surfactant protein A promotes the macrophage-mediated uptake of lung pathogens

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such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.[16] These organisms are also common pathogens in middle ear and paranasal sinus infections.[1,2]

Each SP-A protein molecule consists of an amino terminal type IV collagen–like region and a carboxy terminal region characterized by a globular domain that has sequence homology to mammalian lectins.[15] Surfactant protein A forms trimers that aggregate in groups of 6 to form the SP-A molecule found in purified surfactant, ie, an octadecamer of individual SP-A molecules.[15] Posttranslational modifications of SP-A include glycosylation with N-linked carbohydrates.[15] Surfactant protein A has sequence and structural homology to several other proteins, including the C1q component of complement, surfactant protein D, and mannose-binding protein (MBP).[18] Together, these proteins, all of which are involved in host defense mechanisms, are known as the collectin family of proteins.[18] Mannose-binding protein is secreted by the liver and acts to help clear pathogens from the circulation. It has been reported that mutations in MBP are associated with increased levels of systemic infection in affected individuals.[19] We hypothesize that SP-A, a collectin found in respiratory epithelium, is present in the middle ear and paranasal sinuses and may be an important factor in conferring local immunity in these anatomic sites.

MATERIALS AND METHODS

Tissue. Fifteen adult male rabbits were purchased for this study. Six rabbits were obtained from the Knapp Creek Rabbitry, Amana, Iowa. All of these rabbits were noted to have evidence of acute or chronic sinus and middle ear infections upon direct examination after euthanization. The pathogen in the infected animals was identified by culture to be *Pasteurella multocida*. A high proportion of rabbits harbor this pathogen and show symptoms of infection with stress. Six additional animals obtained from the same source were treated for 7 days with oral tetracycline and had no evidence of acute infection after this treatment. Three rabbits obtained from Harlan Sprague Dawley (Indianapolis, Ind) were reared in a pathogen-free environment. All animals were euthanized by rapid injection of sodium pentobarbital (500 mg) in an ear vein. Tissue samples were obtained by curettage from the maxillary sinuses and the middle ear. Lung, trachea, and liver were also collected from each animal. All tissues were immediately frozen in liquid nitrogen and stored at –70°C.

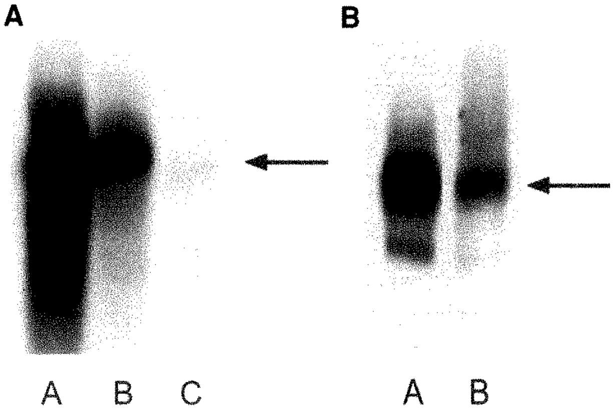

Fig 1. Surfactant protein A (SP-A) messenger RNA (mRNA) in rabbit middle ear and sinus tissues. Total RNA was isolated, separated by gel electrophoresis, transferred to membrane, and probed with radiolabeled rabbit SP-A complementary DNA (cDNA). A) Northern blot analysis of SP-A mRNA in adult rabbit lung (lane A), trachea (lane B), and maxillary sinus (lane C). Ear and sinus in this animal were infected. SP-A mRNA (1.9 kb, arrow) was detected in lung, trachea, and maxillary sinus. B) Northern blot analysis of SP-A mRNA in adult rabbit lung (lane A) and middle ear (lane B). Middle ear was infected in this animal. SP-A mRNA (arrow) was detected in lung and middle ear tissues.

RNA Isolation. Frozen tissue was homogenized in 1 mL of RNA extraction mixture, and total RNA was isolated as described previously.[20] The precipitated RNA pellet was washed with 75% ethanol, air-dried, and resuspended in sterile RNase-free, DNase-free water. RNA was quantified by absorbance at 260 nm, and purification was determined by the ratio of absorbance at 260 to 280 nm. Ten to 20 µg of RNA was electrophoresed on 1.2% agarose/5% formaldehyde gel. A Polaroid photograph of the ethidium bromide–stained gel was obtained under ultraviolet (UV) light. The RNA was transferred by capillary action to a positively charged nylon membrane (Nytran Plus, Schleicher and Schuell, Kenne, NH) and cross-linked to the membrane by UV irradiation in a GS Gene Linker (Bio-Rad, Hercules, Calif).

Northern Blot Analysis. A rabbit complementary DNA (cDNA) probe for SP-A (gift from Dr Carole R. Mendelson[21]) was radiolabeled with [α-^{32}P] deoxycytosine triphosphate (approximately 3,000 Ci/mmol; Amersham, Bedford, Mass) with a random-primer kit (Boehringer Mannheim Biochemicals, Indianapolis, Ind). Prehybridization of the membranes was carried out for 6 hours, and then the labeled cDNA probe was added (1×10^6 cpm/mL) and allowed to hybridize overnight. Membranes were washed and exposed to X-ray film (Eastman Kodak Co, Rochester, NY) with an intensifying screen at –70°C as described previously.[9]

Reverse Transcriptase–Polymerase Chain Reaction. One microgram of RNA was reverse-transcribed

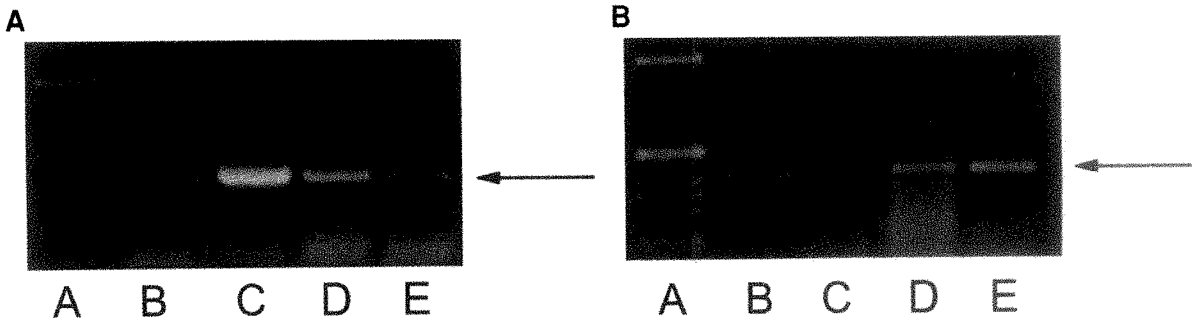

Fig 2. Reverse transcriptase–polymerase chain reaction (RT-PCR) for SP-A mRNA in rabbit middle ear and sinus. One microgram of total RNA from each sample was reverse-transcribed and amplified by PCR. Size of expected PCR product is 387 bp (arrows). A) Rabbit lung contained SP-A mRNA (lane C), while rabbit liver did not (lane B). SP-A mRNA was detected in 2 different rabbit middle ear samples (lanes D and E). B) SP-A mRNA was detected in 2 different rabbit maxillary sinus samples (lanes D and E). Rabbit lung contained SP-A mRNA (lane B), while rabbit liver did not (lane C). One kilobase DNA ladder (Gibco-BRL) was used as molecular weight standard (lane A in both panels).

to cDNA in the presence of RNase inhibitor, oligo dT primer, and murine Maloney leukemia virus reverse transcriptase (RT; Gibco-BRL Life Technologies, Inc, Rockville, Md) in a 30-µL volume for 1 hour at 37°C. Five microliters of the RT reaction was amplified by polymerase chain reaction (PCR) with Amplitaq Polymerase (Perkin-Elmer, Foster City, Calif) and gene-specific oligonucleotides. The following parameters were used for all PCR amplifications: denaturation at 94°C for 10 minutes, followed by 40 cycles that consisted of 30 seconds of denaturation (94°C), 30 seconds of annealing (55°C), and 30 seconds of elongation (72°C). After completion of amplification, the reaction mix was heated for 10 minutes at 72°C. Oligonucleotides used for amplification were as follows — SP-A: sense strand 5'-CTAGACGAGGAGCTCCAAGCCACA-3' (nucleotides 361 to 384) and antisense strand 5'-CCCATCTGTGTACATCTCCACACA-3' (nucleotides 724 to 747, numbering according to Boggaram et al[21]); SP-B: sense strand 5'-GCGGCGTCATGGCCAAGTCACA-3' (nucleotides 2,186 to 2,207) and antisense strand 5'-CTTTGCTGGGAGGGCCCCCAGC-3' (nucleotides 3,688 to 3,709, numbering according to Boggaram and Margana[22]); and SP-C: sense strand 5'- GGGGCCGCTTCGGCATCCCCTG-3' (nucleotides 2,249 to 2,270) and antisense strand 5'-AGCTTGCTTTATTCTCCTGTGG-3' (nucleotides 3,467 to 3,488, numbering according to Boggaram and Margana[23]). Ten microliters of each PCR reaction was electrophoresed on a 1% agarose gel. A Polaroid photograph of the

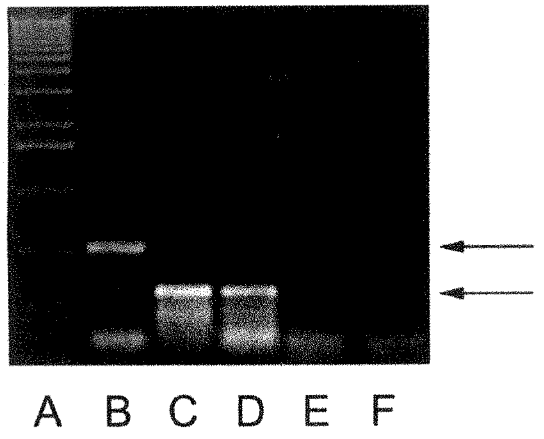

Fig 3. RT-PCR for SP-B mRNA in rabbit middle ear and maxillary sinus. One microgram of total RNA from each sample was reverse-transcribed and amplified by PCR. Size of expected PCR product is 524 bp (upper arrow). Rabbit lung contained SP-B mRNA (lane B), while liver did not (lane E). Approximately 300 bp SP-B mRNA product (lower arrow) was consistently detected in rabbit middle ear (lane C) and maxillary sinus samples (lane D). Lane F — PCR control (no RT substrate). One kilobase DNA ladder (Gibco-BRL) was used as molecular weight standard (lane A).

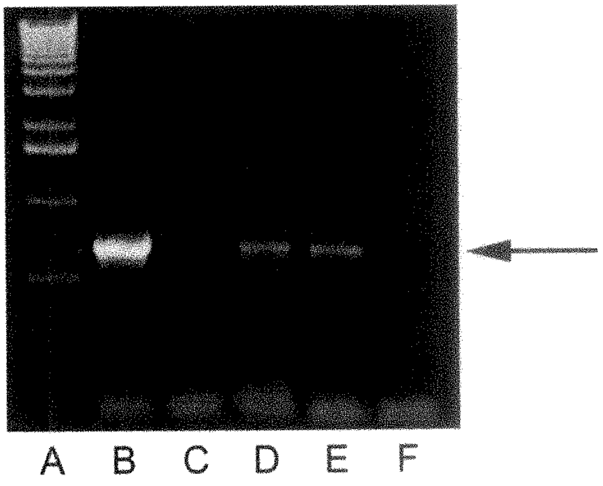

Fig 4. RT-PCR for SP-C mRNA in rabbit middle ear and maxillary sinus. One microgram of total RNA from each sample was reverse-transcribed and amplified by PCR. Size of expected PCR product is 698 bp (arrow). Rabbit lung contained SP-C mRNA (lane B), while liver did not (lane C). SP-C mRNA was detected in some rabbit middle ear (lane D) and maxillary sinus tissues (lane E). Lane F — PCR control (no RT substrate). One kilobase DNA ladder (Gibco-BRL) was used as molecular weight standard (lane A).

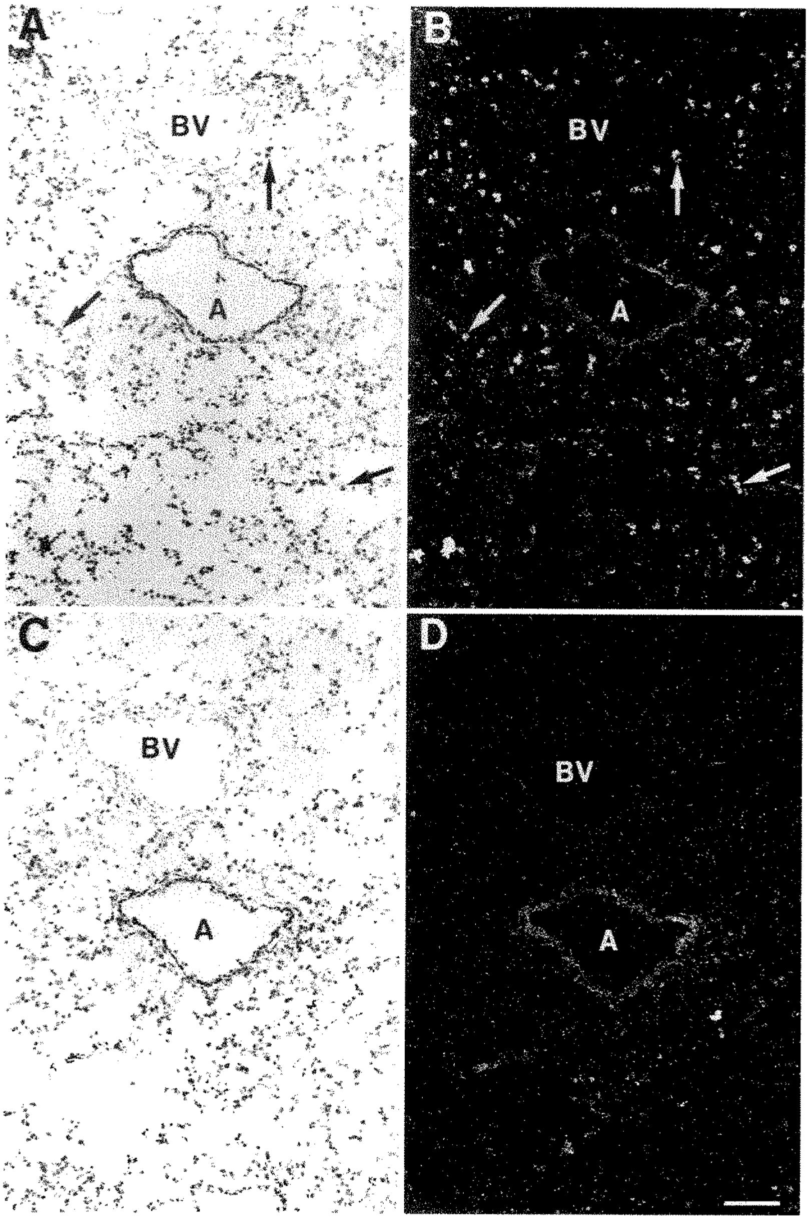

Fig 5. In situ hybridization of SP-A mRNA in adult rabbit lung. Arrows — type II cells, BV — blood vessel, A — conducting airway, bar — 100 µm. A) Bright field microscopy of adult rabbit lung section incubated with [^{3}H]-antisense SP-A complementary RNA (cRNA) probe. B) Dark field microscopy of same area shown in A, with hybridization in alveolar type II cells (arrows). C) Bright field microscopy of adult lung tissue section incubated with [^{3}H]-sense SP-A cRNA probe. D) Dark field microscopy of area shown in D, with no evidence of hybridization.

ethidium bromide–stained gel was obtained under UV light.

In Situ Hybridization. In situ hybridization was carried out as described previously.[24] Seven-micrometer–thick frozen sections were cut at –20°C and mounted on glass slides (Superfrost Plus, Fisher, Chicago, Ill). Sense or antisense [^{3}H] complementary RNA (cRNA) probe (approximately 3×10^5 cpm) in hybridization buffer was applied to each slide as described previously.[24] Coverslips (HybriWell Chambers, Lab Vision Corp, Fremont, Calif) were placed atop the sections and sealed, and the slides were incubated at 60°C overnight in a humid chamber. After removal of coverslips, the slides were rinsed as described previously.[24] The sections were dehydrated through an ethanol series, air-dried, coated with nuclear track (NTB-2) emulsion (Eastman Kodak, Rochester, NY), dried, and exposed in a light-proof box at 4°C. Autoradiograms were developed in D-19 developer, rinsed in distilled water, fixed in Rapid Fixer (Kodak), rinsed in distilled water, stained with hematoxylin, dehydrated, and mounted with glass coverslips. A section of rabbit lung tissue was included on every slide as a control.

Immunoblot Analysis. Tissue was homogenized in sterile water containing phenylmethylsulfonyl fluoride (1 mmol/L), and the homogenate was centrifuged at 600*g* for 10 minutes at 4°C. The supernatant was collected and the protein concentration measured by the method of Bradford.[25] Between 50 and 100 µg of total protein homogenate was separated by electrophoresis on a 10% polyacrylamide minigel (BioRad) with Tris-glycine–sodium dodecyl sulfate (SDS) buffer (BioRad). After separation, the proteins were elec-

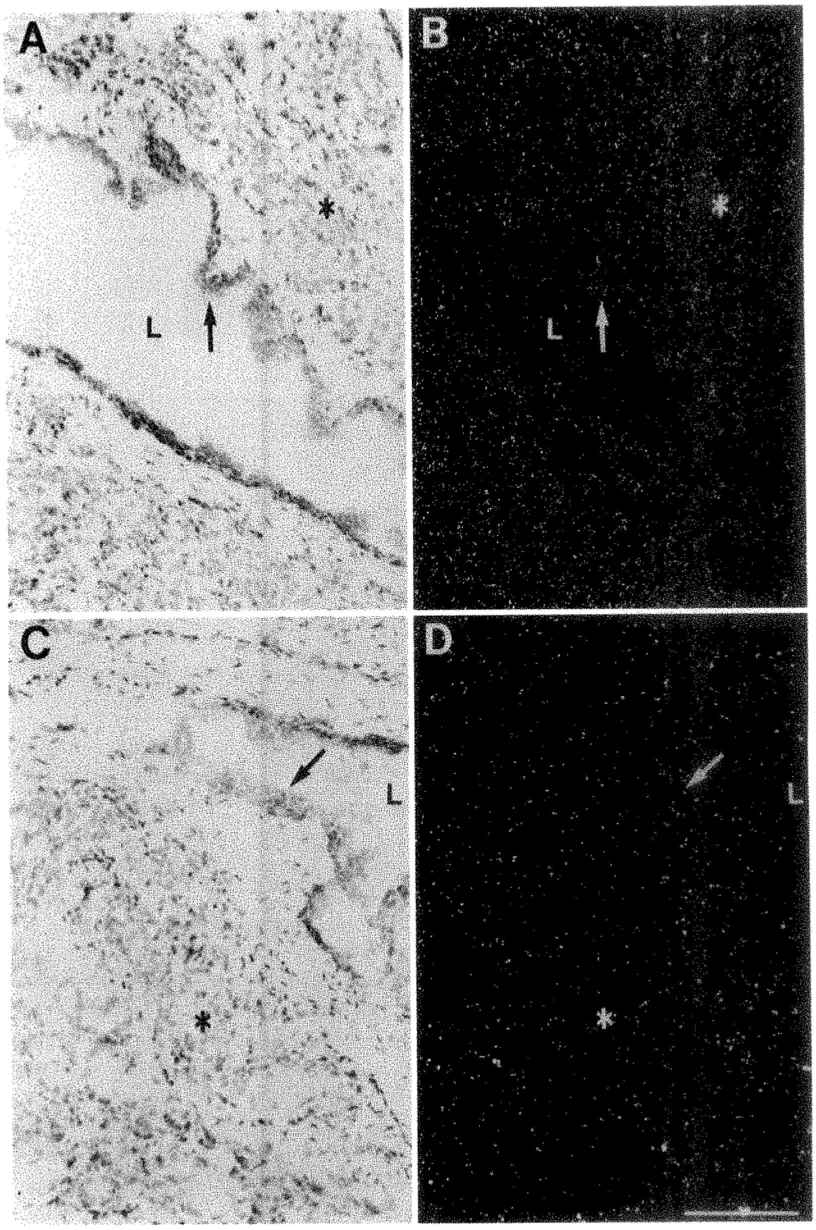

Fig 6. In situ hybridization of SP-A mRNA in maxillary sinus tissues. L — lumen, asterisk — submucosal tissue, arrow — epithelium, bar — 100 μm. A) Bright field microscopy of maxillary sinus tissue section incubated with [^{3}H]-antisense SP-A cRNA probe. B) Dark field image of same area shown in A, demonstrating hybridization in submucosa (asterisk) and lack of specific hybridization to surface epithelium (arrow). C) Bright field microscopy of sinus epithelium hybridized with [^{3}H]-sense SP-A probe. D) Dark field microscopy of area shown in C, showing background hybridization.

trophoretically transferred to Immobilon membranes (Millipore Corp, Bedford, Mass). The unoccupied sites on the membranes were blocked by incubating the membranes in blocking buffer (7% nonfat dry milk in 0.1% Tween 20, 150-mmol/L sodium chloride, and 10-mmol/L Tris-hydrochloride [HCl], pH 7.5) overnight at 4°C. The membranes were then incubated in goat anti-rabbit SP-A antibodies[26] for 1 hour at room temperature with gentle agitation. After five 6-minute rinses in 0.1% Tween 20, 150-mmol/L sodium chloride, and 10-mmol/L Tris-HCl (TNT), the membranes were incubated in rabbit anti-goat IgG conjugated to horseradish peroxidase (Cappel, ICN Pharmaceuticals Inc, 1:5,000 in blocking buffer) for 45 minutes at room temperature. After six 5-minute washes in TNT, the reactive immune complexes were detected by means of enhanced chemiluminescence (Amersham Life Sciences-USB, Arlington Heights, Ill) and blue-sensitive film (Midwest Scientific, St Louis, Mo).

Deglycosylation. Proteins in the homogenates were deglycosylated with the enzyme peptide-N-glycosidase F (PNGF; Boehringer Mannheim).[27] One hundred micrograms of protein was denatured by addition of 2.5 μL of 1-mol/L β-mercaptoethanol/5% SDS in a total volume of 50 μL followed by boiling for 5 minutes. The mixture was cooled and divided equally into 2 tubes. We added 18.5 μL Tris-HCl, pH 8.0, and 6.5 μL 10% Triton X-100 to each tube. One unit of enzyme (1 μL) was added to one tube, while the other tube received 1 μL of Tris buffer (control). Both tubes were incubated overnight at 37°C, and the reaction was stopped by boiling the tubes for 5 min-

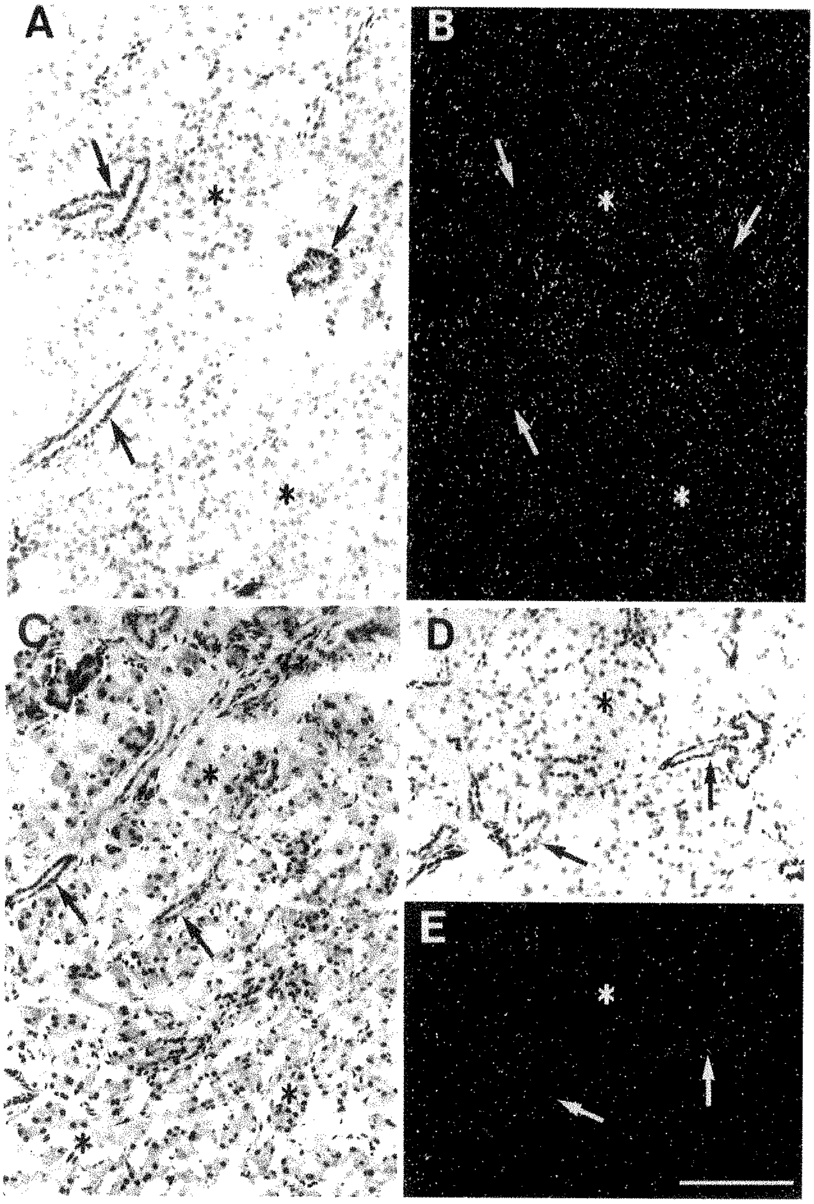

Fig 7. In situ hybridization of SP-A mRNA in maxillary sinus tissue. Arrows — submucosal gland ducts, asterisks — submucosal gland secretory cells, bar — 100 μm. A) Bright field microscopy of maxillary sinus tissue incubated with [^{3}H]-antisense SP-A cRNA probe. B) Dark field microscopy of area shown in panel A, with hybridization in submucosal glands (asterisks). Ducts are indicated with arrows. C) Hematoxylin-stained section of maxillary sinus tissue showing submucosal gland ducts (arrows) and secretory cells (asterisks). D) Bright field microscopy of submucosal gland tissue incubated with [^{3}H]-sense SP-A cRNA probe. E) Dark field microscopy of area shown in panel D, with no evidence of hybridization.

utes. Then, 25 μL of each reaction was analyzed by immunoblotting as described above.

Immunostaining. Seven-micrometer frozen sections of the middle ear, sinus, and lung tissue were mounted on glass slides (Superfrost Plus, Fisher). Sections were fixed for 10 minutes in 10% formalin in phosphate buffered saline (PBS), rinsed in PBS, and immunostained with a Vectastain Elite kit (Vector Labs, Burlingame, Calif) by following the manufacturer's protocol. After blocking of nonspecific sites with normal goat serum, slides were incubated for 1 hour in guinea pig anti-human SP-A antibodies.[9] The tissue sections were rinsed with PBS and incubated in biotinylated secondary antibody for 30 minutes and then rinsed and incubated in avidin-peroxidase reagent for 45 minutes. After rinsing, sections were incubated in substrate, ie, 0.7 mg/mL diaminobenzidine (Sigma, St Louis, Mo) in PBS. Sections were rinsed, counterstained with hematoxylin, dehydrated, and mounted with coverslips. Sections were photographed with a Nikon FX photomicroscope (Nikon, Melville, NY).

RESULTS

Surfactant protein A messenger RNA (mRNA) was detected in adult rabbit lung, trachea, middle ear, and maxillary sinus tissues by Northern blot analysis (Fig 1). It was detected in the middle ear and/or sinus tissues of 7 of 13 animals examined by Northern blot analysis. The size of the SP-A mRNA transcript was the same in all of the tissues. However, the amount of SP-A mRNA was almost always less in the middle ear and sinus tissues as compared to levels in the lung.

Reverse transcriptase–PCR revealed the presence

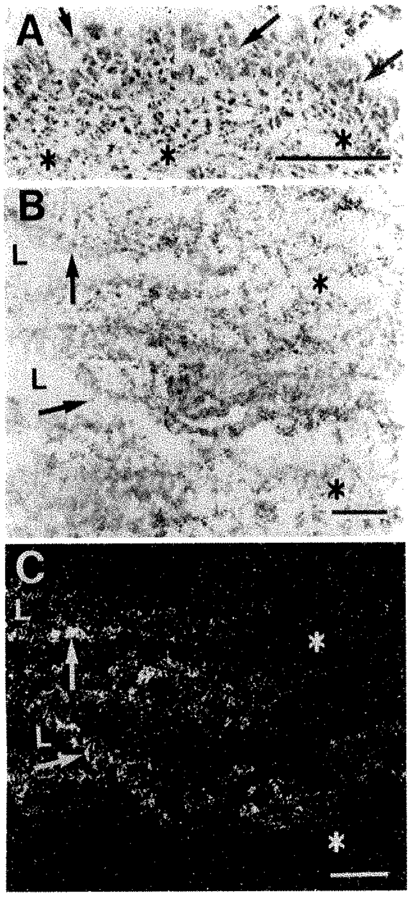

Fig 8. In situ hybridization of SP-A mRNA in middle ear tissues. L — lumen, arrows — epithelium, asterisks — submucosa, bars — 100 µm. A) Hematoxylin-stained section of middle ear tissue. No submucosal glands were observed in this sample. B) Bright field microscopy of middle ear tissue incubated with [^{3}H]-antisense SP-A cRNA probe. C) Dark field microscopy of area shown in panel A, with hybridization present in epithelium and absent in submucosa.

of SP-A, SP-B, and SP-C mRNAs in the rabbit maxillary sinus and middle ear (Figs 2-4). The SP-A mRNA was detected by RT-PCR in middle ear and maxillary sinus, thus confirming our Northern blot observations (Fig 2). We were able to detect SP-A mRNA in sinus tissues in 12 of 15 animals evaluated and in the middle ear tissue of 6 of 8 animals examined. The SP-B mRNA was detected in 12 of 15 sinus tissues and 9 of 10 middle ear tissues (Fig 3). The size of the SP-B RT-PCR product was smaller than that observed in the lung. The SP-C mRNA was detected in a few (4 total) middle ear and/or maxillary sinus tissue samples (Fig 4).

In situ hybridization was used to detect intracellular SP-A mRNA in lung, maxillary sinus, and middle ear tissues. The SP-A mRNA was detected in adult rabbit lung tissue primarily in alveolar type II cells (Fig 5A,B). In control slides, incubated with the sense cDNA probe, only background grains were observed over the lung tissue (Fig 5C,D). Surfactant protein A mRNA was also detected in both the rabbit maxillary sinus and middle ear tissues (Figs 6-8). The maxillary sinus tissue contained large numbers of submucosal glands (Fig 7C). The SP-A mRNA was primarily present in epithelial cells of the submucosal glands (Figs 6 and 7). In contrast, in the middle ear tissues, the SP-A mRNA was present primarily in the surface epithelium (Fig 8). There were few glands in the middle ear submucosal tissue (Fig 8).

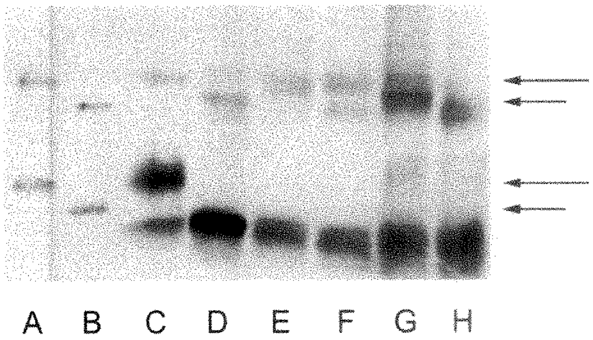

Fig 9. Immunoblot analysis of SP-A protein in rabbit lung, middle ear, and maxillary sinus tissues. Human SP-A purified from alveolar proteinosis material was also examined. Protein homogenates of lung, middle ear, and maxillary sinus tissues were separated by polyacrylamide gel electrophoresis, transferred to membrane, and then probed with polyclonal goat anti-rabbit SP-A antibodies. Approximately 35 kd SP-A monomer and approximately 70 kd SP-A dimer were detected in human alveolar proteinosis material (lane A) and rabbit lung tissue (lane C) and are indicated by lower and upper long arrows, respectively. Approximately 29 kd SP-A–immunoreactive band was detected in rabbit middle ear (lane E) and in rabbit maxillary sinus (lane G). Deglycosylation of SP-A by peptide-N-glycosidase-F (PNGF), enzyme that cleaves N-linked carbohydrate residues, resulted in lower–molecular weight human and rabbit SP-A–immunoreactive bands (lanes B and D, upper and lower short arrows). In contrast, molecular weight of SP-A in rabbit middle ear and sinus was not altered by PNGF digestion (lanes F and H, respectively).

Immunoblot analysis revealed the presence of SP-A protein in rabbit maxillary sinus and middle ear tissues (Fig 9). It was detected as approximately 70 kd, 35 kd, and 29 kd bands in adult rabbit lung tissue (Fig 9). Human alveolar proteinosis SP-A was detected as an approximately 35 kd monomer and an approximately 70 kd dimer (Fig 9). The SP-A protein migrated at a molecular weight of approximately 29 kd in rabbit middle ear and maxillary sinus tissues (Fig 9). In the distal lung, SP-A protein has been shown to be modified by carbohydrates that can be cleaved with PNGF, an enzyme that cleaves N-linked oligosaccharides.[27] An immunoreactive SP-A band of reduced molecular weight (approximately 29 kd) was observed in purified human alveolar proteinosis SP-A and adult rabbit lung tissue SP-A after deglyco-

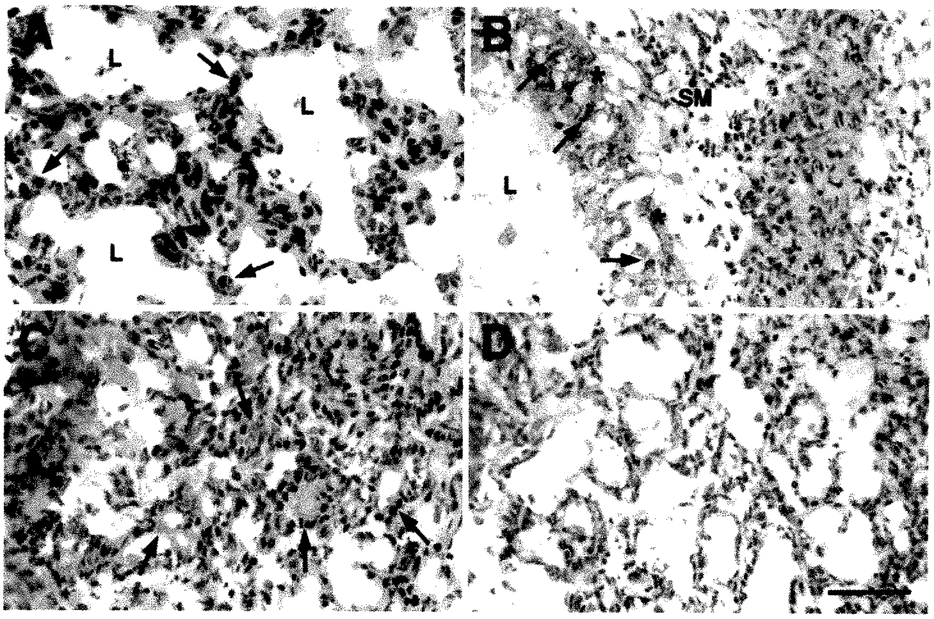

Fig 10. SP-A immunostaining of lung, middle ear, and maxillary sinus tissues. Arrows — brown staining for SP-A, SM — submucosal tissue, L — lumen, bar — 100 μm. A) SP-A was present in alveolar type II cells (arrows) of adult rabbit lung tissue. B) SP-A was localized in surface epithelium (arrows) of rabbit middle ear tissues. Asterisks — epithelium. C) SP-A was localized in submucosal glands (arrows) of maxillary sinus tissues. D) No staining was observed in maxillary sinus tissues when phosphate-buffered saline was used instead of primary antibody.

sylation (Fig 9). In contrast, the molecular weight of the SP-A in middle ear and sinus tissues was not affected by PNGF digestion (Fig 9).

Immunostaining for SP-A protein was performed on frozen sections of lung, maxillary sinus, and middle ear tissues (Fig 10). The SP-A was detected in alveolar type II cells in the adult rabbit lung tissue (Fig 10A). In the middle ear, most of the SP-A immunoreactivity was detected in the surface epithelium (Fig 10B). Immunostaining of the maxillary sinus tissues demonstrated evidence of the SP-A protein primarily in the submucosal glands (Fig 10C). No staining was observed in sinus tissues immunostained with PBS instead of the primary antibody (Fig 10D).

DISCUSSION

Although often considered nuisance diseases, sinusitis and otitis media have a significant impact both on the individual suffering from the disease process and on the health care system as a whole.[1,2] The pathophysiologies of sinusitis and otitis media are not well understood. While it is well recognized that systemic immunity plays a role in recurrent or chronic sinusitis and otitis media, little attention has been given to the role of local immunity in the causation of sinusitis and otitis media.

Surfactant protein A is a lung protein known to play an important role in local defense mechanisms against bacteria and viruses at the level of the alveolus.[16] It is a member of the collectin family, proteins that mediate local defense against pathogenic microorganisms by acting as opsonins, increasing the uptake of pathogens by macrophages, and activating the respiratory burst in macrophages.[16,18] Although SP-A in the distal lung is synthesized primarily by type II pneumocytes, SP-A has also been shown to be present in the respiratory epithelium and submucosal glands of the conducting airways,[7-9] and there is evidence to suggest that it may be present in the respiratory epithelium of the eustachian tube and middle ear.[3,4] The paranasal sinuses are also lined by respiratory epithelium; however, SP-A has not previously been described in this location.[6] The data presented in this study confirm the presence of SP-A in the middle ear and suggest that SP-A is also present in the paranasal sinuses.

Surfactant protein A mRNA levels were much lower in the maxillary sinus and middle ear tissues than in the lung, as shown by the density of reactive bands in Northern blot analysis. In addition, more middle ear and sinus samples were positive by RT-PCR, a more sensitive technique, than by Northern blot analysis. The size of the SP-A mRNA transcript was the same in the lung, trachea, middle ear, and sinus tissues. In general, SP-A mRNA was either undetectable or present in very low amounts in middle ear and sinus tissues from pathogen-free rabbits. In addition, there did not seem to be a difference in the amount of SP-A mRNA in infected animals versus those treated with antibiotics. We did not quantify the amount of SP-A mRNA in this study and therefore cannot directly address whether infection alters SP-A mRNA levels in the rabbit middle ear or paranasal sinus. Other investigators have shown that SP-A levels in bronchoalveolar lavage fluid are reduced in patients with pneumonia.[28]

An approximately 29 kd SP-A protein was detected in middle ear and sinus tissues by immunoblot analysis. This corresponds to the molecular weight of the unglycosylated SP-A molecule.[26] Another prominent SP-A–immunoreactive band was detected in the middle ear and sinus samples at approximately 70 kd. Interestingly, the relative proportion of the higher–molecular weight SP-A molecule was greater in the middle ear and sinus samples than in the lung. Digestion with PNGF reduced the molecular weight of lung SP-A, but had relatively little effect on the molecular weight of the SP-A in the middle ear and sinus. Together, these data are suggestive that the SP-A in the middle ear and sinus tissues is not posttranslationally modified in the same manner as the SP-A protein in the distal lung.

Surfactant protein A mRNA and protein were localized by in situ hybridization and immunostaining, respectively, in the epithelium of the middle ear. These observations confirm the previous results of Yamanaka et al[3] and Kobayashi et al.[4] We also localized SP-A mRNA and protein in the maxillary sinus, specifically in submucosal glands. Surfactant protein A has also been detected in fetal and adult human conducting airway submucosal glands.[7-9] The results of our in situ hybridization and immunostaining studies were in agreement and are suggestive that SP-A is expressed in different sites in the middle ear and paranasal sinuses.

Both SP-B mRNA and SP-C mRNA were also detected in the middle ear and sinus tissues. Interestingly, the SP-B PCR product was smaller in both the middle ear and sinus tissue than in the lung. The existence of an alternatively spliced SP-B mRNA has been reported in mouse and rabbit lung tissue.[29] The alternatively spliced lung SP-B mRNA transcript, which constitutes as much as 30% of all SP-B mRNA in the mouse lung, has a 69 bp deletion in exon 7.[29] This region was not included in the PCR product examined in the present study; therefore, yet another transcript of the SP-B gene may exist. We have not further characterized SP-B mRNA expressed in the middle ear and sinus. Surfactant protein C mRNA was also detected in the middle ear and sinus tissues by RT-PCR, although with much less frequency than either SP-B or SP-A mRNA. The existence of the surfactant protein mRNAs in the middle ear and sinus tissues, combined with previous reports concerning the presence of surfactant phospholipids and surface tension–lowering properties of washes obtained from the middle ear and paranasal sinuses, is suggestive that surfactant systems exist in these sites that are similar but not identical to those in the lung.[12-14]

REFERENCES

1. Evans KL. Diagnosis and management of sinusitis. Br Med J 1994;309:1415-22.

2. Haddad J Jr. Treatment of acute otitis media and its complications. Otolaryngol Clin North Am 1994;27:431-41.

3. Yamanaka N, Kobayashi K, Kataura A, Kuroki Y, Akino T. Implication of surfactant apoprotein in otitis media with effusion. Ann Otol Rhinol Laryngol 1991;100:835-40.

4. Kobayashi K, Yamanaka N, Kataura A, Ohtani S, Saito T, Akino T. Presence of an 80 kilodalton protein, cross-reacted with monoclonal antibodies to pulmonary surfactant protein A, in the human middle ear. Ann Otol Rhinol Laryngol 1992;101:491-5.

5. Lim DJ. Functional morphology of the lining membrane of the middle ear and eustachian tube. An overview. Ann Otol Rhinol Laryngol Suppl 1974;83(suppl 11):5-26.

6. Junqueira LC, Carneiro J, Kelley RO. Basic histology. Norwalk, Conn: Appleton and Lange, 1995:325-45.

7. Khoor A, Gray ME, Hull WM, Whitsett JA, Stahlman MT. Developmental expression of SP-A and SP-B mRNA in the proximal and distal respiratory epithelium of the human fetus and newborn. J Histochem Cytochem 1993;41:1311-9.

8. Saitoh H, Okayama H, Shimura S, Fushimi T, Masuda T, Shirato K. Surfactant protein A2 gene expression by human airway submucosal gland cells. Am J Respir Cell Mol Biol 1998;19:202-9.

9. Goss KL, Kumar AR, Snyder JM. SP-A2 gene expression in human fetal lung airways. Am J Respir Cell Mol Biol 1998;19:613-21.

10. Wright JR, Clements JA. Metabolism and turnover of lung surfactant. Am Rev Respir Dis 1987;136:426-44.

11. Avery ME, Mead J. Surface properties in relation to atelectasis and hyaline membrane disease. Am J Dis Child 1949;97:517-23.

12. Birkin EA, Brookler KH. Surface tension lowering substance of the canine eustachian tube. Ann Otol Rhinol Laryngol 1972;81:268-71.

13. Hills BA. Analysis of eustachian surfactant and its function as a release agent. Arch Otolaryngol 1984;110:3-9.

14. Grace A, Kwok P, Hawke M. Surfactant in middle ear effusions. Otolaryngol Head Neck Surg 1987;96:336-40.

15. McCormack FX. Structure, processing and properties of surfactant protein A. Biochim Biophys Acta 1998;1408:109-31.

16. Crouch EC. Collectins and pulmonary host defense. Am J Respir Cell Mol Biol 1998;19:177-201.

17. Tenner AJ, Robinson SL, Borcheit J, Wright JR. Human pulmonary surfactant protein (SP-A), a protein structurally homologous to C1q, can enhance FcR and CR1-mediated phagocytosis. J Biol Chem 1989;264:13923-8.

18. Reid KB. Structure/function relationships in the collectins (mammalian lectins containing collagen-like regions). Biochem Soc Trans 1993;21:464-8.

19. Summerfield JA, Ryder S, Sumiya M, et al. Mannose binding protein gene mutations associated with unusual and severe infections in adults. Lancet 1995;345:886-9.

20. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156-9.

21. Boggaram V, Qing K, Mendelson CR. The major apo-

protein of rabbit pulmonary surfactant: elucidation of primary sequence and cAMP and developmental regulation. J Biol Chem 1988;263:2939-47.

22. Boggaram V, Margana RK. Rabbit surfactant protein B gene: structure and functional characterization of the promoter. Am J Physiol 1996;270:L601-L612.

23. Boggaram V, Margana RK. Rabbit surfactant protein C: cDNA cloning and regulation of alternatively spliced surfactant protein C mRNAs. Am J Physiol 1992;263:L634-L644.

24. Wohlford-Lenane CL, Snyder JM. Localization of surfactant-associated proteins SP-A and SP-B in rabbit fetal lung tissue by in situ hybridization. Am J Respir Cell Mol Biol 1992;7: 335-43.

25. Bradford MM. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.

26. Snyder JM, Mendelson CR. Induction of the major surfactant apoprotein during rabbit fetal lung development. Biochim Biophys Acta 1987;920:226-36.

27. Lemp D, Haselbeck A, Klebl F. Molecular cloning and heterologous expression of N-glycosidase F from *Flavobacterium meningosepticum*. J Biol Chem 1990;265:15606-10.

28. Baughman RP, Sternberg RI, Hull W, Buschbaum JA, Whitsett JA. Decreased surfactant protein A in patients with bacterial pneumonia. Am Rev Respir Dis 1993;147:653-7.

29. Chi X, Garnier G, Hawgood S, Cotton HR. Identification of a novel alternatively spliced mRNA of murine pulmonary surfactant protein B. Am J Respir Cell Mol Biol 1998;19:107-13.

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CERVICAL VERTEBRAL ANOMALIES IN PATIENTS WITH ANOMALIES OF THE HEAD AND NECK

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Congenital head and neck anomalies can occur in association with vertebral anomalies, particularly of the cervical vertebrae. While the former are easily recognized, especially when part of a syndrome, the latter are often occult, thereby delaying their diagnosis. The presence of vertebral anomalies must be considered in pediatric patients with head and neck abnormalities to expedite management of select cases and to prevent neurologic injury. We present our experience with 5 pediatric patients who were referred to the Department of Otolaryngology–Head and Neck Surgery at the University of Iowa with a variety of syndromic anomalies of the head and neck. Each patient was subsequently also found to have a vertebral anomaly. The relevant embryogenesis of the anomalous structures is discussed, with highlighting of potential causes such as teratogenic agents and events and germ-line mutations. A review of syndromes having both head and neck and vertebral anomalies is presented to heighten awareness of otolaryngologists evaluating children with syndromic disorders. Finally, the findings on radiographic imaging studies, particularly computed tomography, are discussed to facilitate the prompt diagnosis of vertebral anomalies.

KEY WORDS — cervical vertebral anomalies, craniofacial anomalies.

INTRODUCTION

Infants and children with craniofacial anomalies often have associated abnormalities of the cervical spine. While many of the syndromic associations are well recognized, such as atlantoaxial instability in Down syndrome, scoliosis in Larsen's syndrome, and fused or cervical hemivertebrae in Klippel-Feil anomaly, Goldenhar (oculo-auriculo-vertebral) spectrum, and Apert, Crouzon, and VATER (vertebral defects, imperforate anus, tracheoesophageal fistula, and radial and renal dysplasia) syndromes, the association of vertebral anomalies with other syndromes, such as fetal alcohol syndrome, is less well recognized (see Table[1]).

Coexistent anomalies of the cervical spine and head and neck may arise due to errors of morphogenesis or due to teratogenic insults that affect both developing systems. Since craniofacial anomalies fall under the purview of the otolaryngologist, an awareness of coexistent vertebral anomalies is required, particularly if patients are scheduled for procedures requiring the induction of general anesthesia and/or neck flexion or extension. The following cases illustrate the diverse ways in which pediatric patients with vertebral anomalies present to the otolaryngologist and stress the importance of considering the presence of vertebral anomalies when evaluating infants and children with head and neck anomalies.

CASE REPORTS

Case 1: Klippel-Feil Anomaly. The first patient, the product of an uncomplicated pregnancy, was delivered vaginally without known trauma. She was noted to have congenital hip dysplasia, and at approximately 1 month of age was found to have left torticollis and started on physical therapy. At 3 months of age, an otolaryngological referral was made by the physical therapist, who noted respiratory problems and perioral cyanosis with the onset of neck exercises.

Physical examination confirmed the presence of severe left head tilt with superior rotation of the chin to the right (Fig 1A). There was also mild asymmetry of the face. The entire left sternocleidomastoid muscle was firm and larger than the right, although a discrete mass was not palpable. The remainder of the head, neck, and neurologic examination findings were normal, including the position of her posterior hairline.

Computed tomography (CT) of the vertebral column showed incomplete ossification of the anterior arch of the atlas (C1), lack of fusion of the posterior arch, and rotation of C1 with relationship to the skull

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COMMON SYNDROMES WITH OTOLARYNGOLOGICAL AND VERTEBRAL ANOMALIES

Syndrome or Sequence	*Otolaryngological Findings*	*Vertebral Anomalies*
Down	Epicanthal folds, macroglossia, fissured tongue, short neck, characteristic facies, flat nasal bridge, auricular anomalies, narrow palate	Occipital-atlanto-axial instability
Klippel-Feil	Short neck, with limited movement and webbing (flared trapezius muscle), low posterior hairline, sensorineural or congenital hearing loss 25% to 50%, cleft palate 17%	Cervical and/or thoracic fusion with or without scoliosis, cervical ribs, Sprengel's deformity
Apert's (acrocephalosyndactyly)	Wide nasal root extending into midline cervical defect, craniosynostosis, beak nose, cleft palate, stapedial fixation, prominent supraorbital ridges, orbital hypertelorism, shallow orbits, hypoplastic midface with relative mandibular prognathism, syndactyly	Cervical fusion (usually C5 and C6), progressive calcification, foramen magnum stenosis secondary to Chiari malformation
Crouzon's (craniofacial dysostosis)	Craniosynostosis, maxillary hypoplasia, shallow orbits, proptosis, occasional cleft lip and palate, conductive hearing loss	Cervical fusion (usually C2 and C3 and/or C5 and C6), foramen magnum stenosis secondary to Chiari malformation
Turner's	Pterygium colli, auricular anomalies, epicanthal folds, ptosis, low posterior hairline, occasional cleft palate or high arched palate, cervical cystic hygromas	Cervical hypoplasia
Oculo-auriculo-vertebral spectrum (Goldenhar's)	Hemifacial microsomia, epibulbar dermoids, colobomas, atresia, microtia, preauricular skin tags, conductive or sensorineural hearing loss, facial nerve anomalies, micrognathia, macrostomia, cleft lip and/or palate	Cervical fusion, hemivertebrae, butterfly vertebrae, hypoplastic vertebrae, Chiari malformation, spina bifida, scoliosis, anomalous ribs
VATER	Tracheoesophageal fistula with esophageal atresia	Cervical segmentation defects
Wildervanck (cervico-oculo-acoustic)	Klippel-Feil anomaly with sensorineural hearing loss, sixth nerve palsy, retracted globe, preauricular skin tags, malformed pinnae, fused ribs	Cervical fusion, hemivertebrae, scoliosis
Achondroplasia	Large calvaria with frontal bossing, low nasal bridge, midface hypoplasia, conductive or sensorineural hearing loss	Invagination of C1 into foramen magnum with cervicomedullary compression, narrowed interpediculate distances
Larsen's	Midface retrusion, depressed and broad nasal bridge, congenital joint dislocations, congenital or sensorineural hearing loss	Cervical instability, abnormal segmentation and/or hypoplasia of cervical and thoracic vertebrae, cervical kyphosis, thoracic scoliosis, flattened vertebral bodies
Mucopolysaccharidoses	Coarse facial features, prominent frontal bones, synophrys, depressed nasal bridge with broad tip and wide nostrils, short neck, macroglossia, mandibular prognathism	Vertebral anomalies prevalent in types I, II, IV, VI, and VII, cervical instability, dysplastic vertebrae, os odontoideum
Gorlin	Nevoid basal cell carcinomas, particularly on face, odontogenic keratocysts, intracranial calcification, increased calvaria, prominent supraorbital ridges, rare cleft lip and/or palate, palmar pits, hypoplastic or bifid ribs	Cervical and/or thoracic fusion
Osteogenesis imperfecta	Congenital and/or sensorineural hearing loss, blue sclerae, opalescent teeth	Vertical movement of upper cervical vertebrae with secondary basilar invagination, vertebral fractures, scoliosis
Ehlers-Danlos	Hyperextensible skin, joint hypermobility, easy bruising	Cervical ligament instability
Fetal alcohol	Microcephaly, short palpebral fissures, ptosis, epicanthal folds, short and upturned nose with broad and low bridge, long and smooth philtrum, midface hypoplasia, mild micrognathia, thin upper lip vermilion border, congenital or sensorineural hearing loss	Cervical fusion (usually C2 and C3)

For comprehensive list of all anomalies of each syndrome, see reference 1.

base (Fig 1B,C). Posterior segmentation defects of C2 and C3 were also noted. The diagnosis of Klippel-Feil anomaly was made, and the patient was fitted with a soft cervical collar. A renal ultrasound study excluded the MURCS association (müllerian duct aplasia, renal aplasia, and cervicothoracic somite dys-

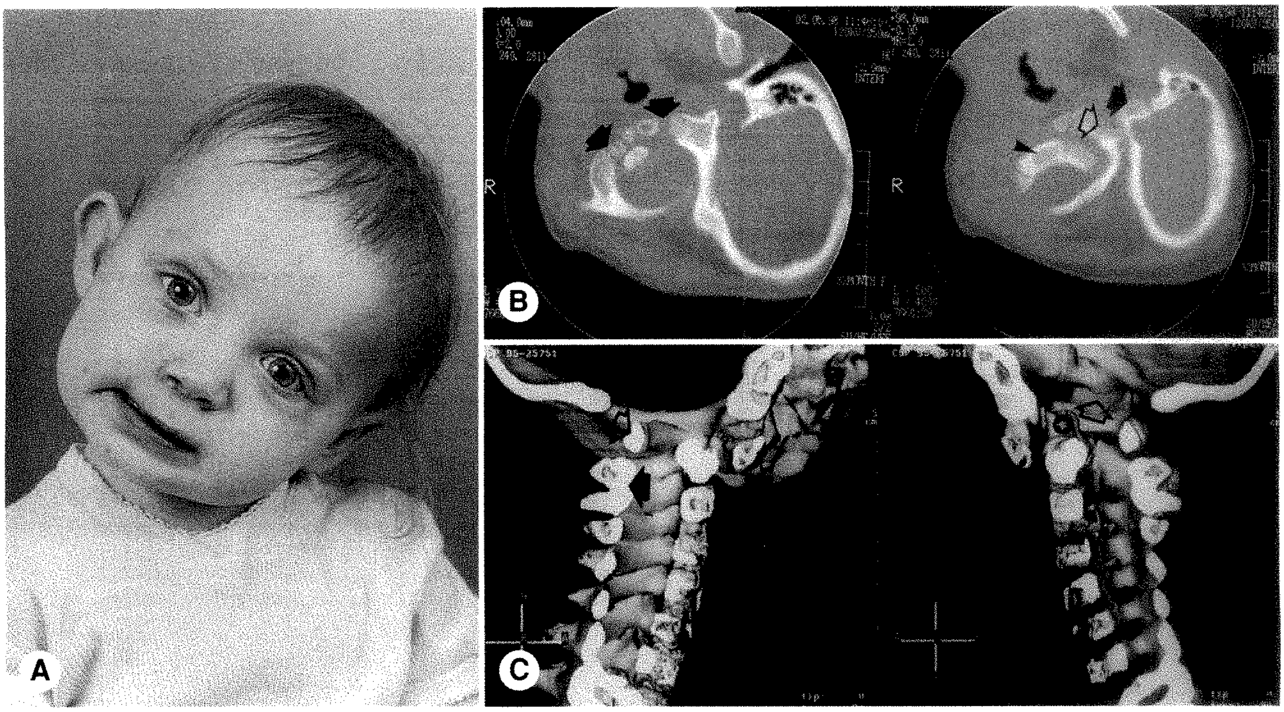

Fig 1. (Case 1) A) Infant with severe torticollis referred for evaluation when physical therapy failed to improve head tilt. Infant was subsequently found to have Klippel-Feil anomaly and associated vertebral anomalies. B) Composite of axial computed tomography (CT) scans through plane of foramen magnum and atlas (left) and atlantoaxial articulation (right). Anterior arch of C1 is incompletely ossified (arrows, left). Left lateral mass of C1 (open arrow, right) has shifted from under occipital condyle (solid arrow, right), causing patient's severe head tilt. Vertical migration of both C1 and C2 (arrowhead, right) is evident, as components of both structures are noted to project into foramen magnum. C) Composite of 3-dimensional CT bony sagittal reconstructions viewing medial and left aspects (left) and medial and right aspects (right) of craniovertebral junction. Note shifting of C1 (open arrow, left) relative to skull base and C2 and posterior segmentation defect of C2 and C3 (solid arrow, left). On mirror image, note medial shift of lateral mass of C1 (open arrow, right) and vertical migration of odontoid process of C2 (star, right).

plasia); a hearing screen yielded normal findings. At 17 months of age, the infant continues to have severe head tilt, but her neurologic examination remains nonfocal. Surgery of the craniovertebral junction is scheduled.

Case 2: Goldenhar's Spectrum. A 14-year-old girl with bilateral grade 3 microtia, atresia, hemifacial microsomia, severe retrognathia, and malocclusion (Fig 2) had had a ventriculoperitoneal shunt placed by a local neurosurgeon when she was an infant, but regular follow-up visits had not been completed. The patient presented as a teenager to the Department of Otolaryngology–Head and Neck Surgery for consideration of surgical repair of her microtia and malocclusion. Vertebral imaging studies were ordered due

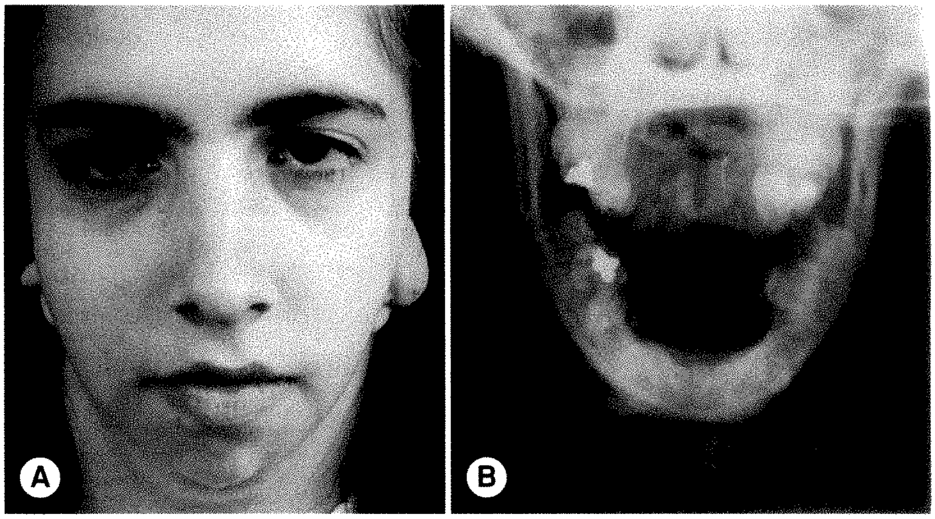

Fig 2. (Case 2) A) Fourteen-year-old girl with Goldenhar's syndrome with bilateral microtia, hemifacial microsomia, and retrognathia. B) Cervical spine frontal radiograph shows abnormal neck position secondary to craniocervical fusion defects.

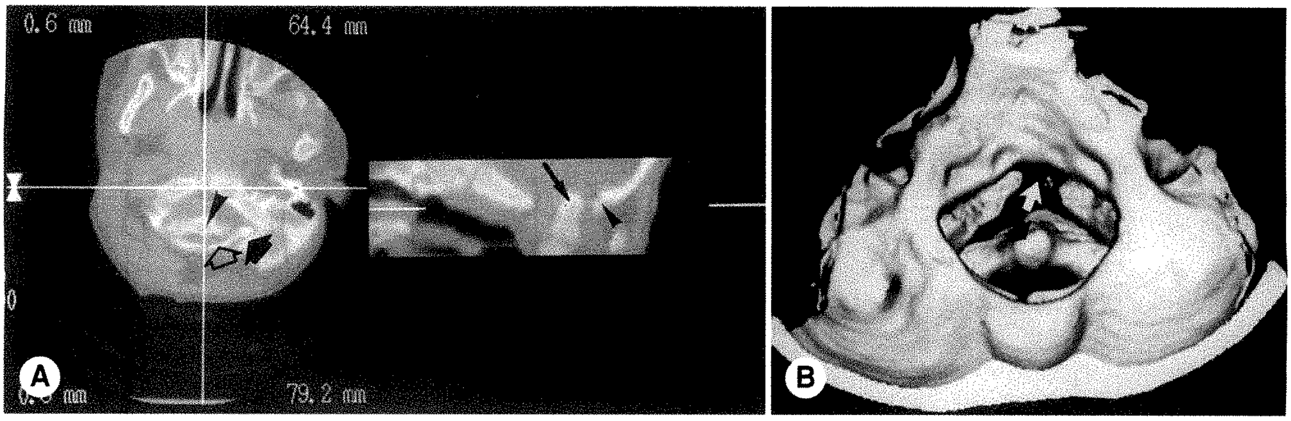

Fig 3. (Case 3) A) Composite of axial CT through craniocervical junction (left) and reformatted sagittal CT of craniocervical junction (right) in 7-year-old boy with trisomy 21 and severe atlantoaxial subluxation. Posterior arch of C1 is upwardly rotated (open arrow, left) such that it lies in same horizontal plane as posterior rim of foramen magnum, also known as ex-occiput (solid arrow, left). Note also that odontoid process of C2 (arrowhead, left) is displaced posteriorly and into foramen magnum. Midsagittal reconstruction also demonstrates upward rotation of C1 (arrowhead, right) and odontoid process (arrow, right) into foramen magnum. B) Three-dimensional CT bone reconstruction of craniovertebral junction, demonstrating absence of anterior arch of C1 (white arrow) and posteriorly displaced odontoid process of C2 (black arrow) markedly reducing diameter of spinal canal.

to the high association of vertebral anomalies with hemifacial microsomia and Goldenhar's spectrum. Cervical films showed diffuse multilevel segmentation anomalies, including hemivertebra and butterfly vertebra and fusion defects at the levels of C3 and C4, C5 and C6, and C7 through T1 (Fig 2). Partial atlas assimilation with atlantoaxial dislocation was also present, and the second vertebra was situated within the spinal canal. The patient was referred to the Neurosurgical Service, and it was recommended that magnetic resonance imaging be performed to help determine the patient's management.

Case 3: Trisomy 21. A male child with trisomy 21 was referred to the Department of Neurosurgery at 7 years of age for the management of C1-C2 subluxation. The diagnosis of subluxation was established incidentally by the child's local otolaryngologist, to whom he had been referred for a hearing evaluation. The parents related the diagnosis of a possible seizure disorder approximately 1 year prior, when the child collapsed, became cyanotic, and was unable to move. This history prompted the astute otolaryngologist to image the cervical spine, documenting severe atlantoaxial subluxation with cervicomedullary compression (Fig 3). The effective spinal canal diameter was 6 mm during neck extension and only 3 mm during neck flexion. These findings were thought to account for the brief episode of "collapse," which was likely followed by a period of acute traumatic quadriparesis. It is interesting to note that flexion and extension films obtained at 4 years of age were reportedly normal.

After reduction with halo traction and vest immobilization, the patient underwent posterior decompression of the foramen magnum, removal of the posterior arch of C1, and fusion of the occiput to C2 and C3. He recovered well postoperatively and was maintained in halo immobilization for 5 months, at which time bony union of his fusion site was confirmed radiographically.

Case 4: Cleft Lip. A 3-month-old boy presented to the Craniofacial Clinic in the Department of Otolaryngology–Head and Neck Surgery for evaluation of a cleft lip deformity. By history, both the pregnancy and vaginal delivery were uncomplicated. Physical examination revealed an incomplete cleft of the left lip, with an intact alveolus and palate. The remainder of the head and neck examination yielded unremarkable findings, with the exception of mild orbital asymmetry, which prompted evaluation for coexistent craniosynostosis. The cranium appeared normal on skull films, but absence of the anterior arch of C1 was noted. Computed tomography of the cervical vertebrae confirmed these findings and also disclosed absence of the posterior arch of C1 and a bifid posterior arch of C2 (Fig 4). The patient was evaluated by the Neurosurgical Service and fitted with a soft cervical collar.

At 9 months of age, the patient underwent repair of his cleft lip and was intubated fiberoptically for the procedure. At 10 months of age, cervical radiographs showed normal vertebral alignment but absence of an ossified anterior arch of C1. The patient's neurologic development is satisfactory.

Case 5: Larsen's Syndrome. A 7-year-old boy with Larsen's syndrome had had a tracheotomy at age 15 months for severe tracheomalacia and bronchomalacia. At age 4 years, the patient was weaned from

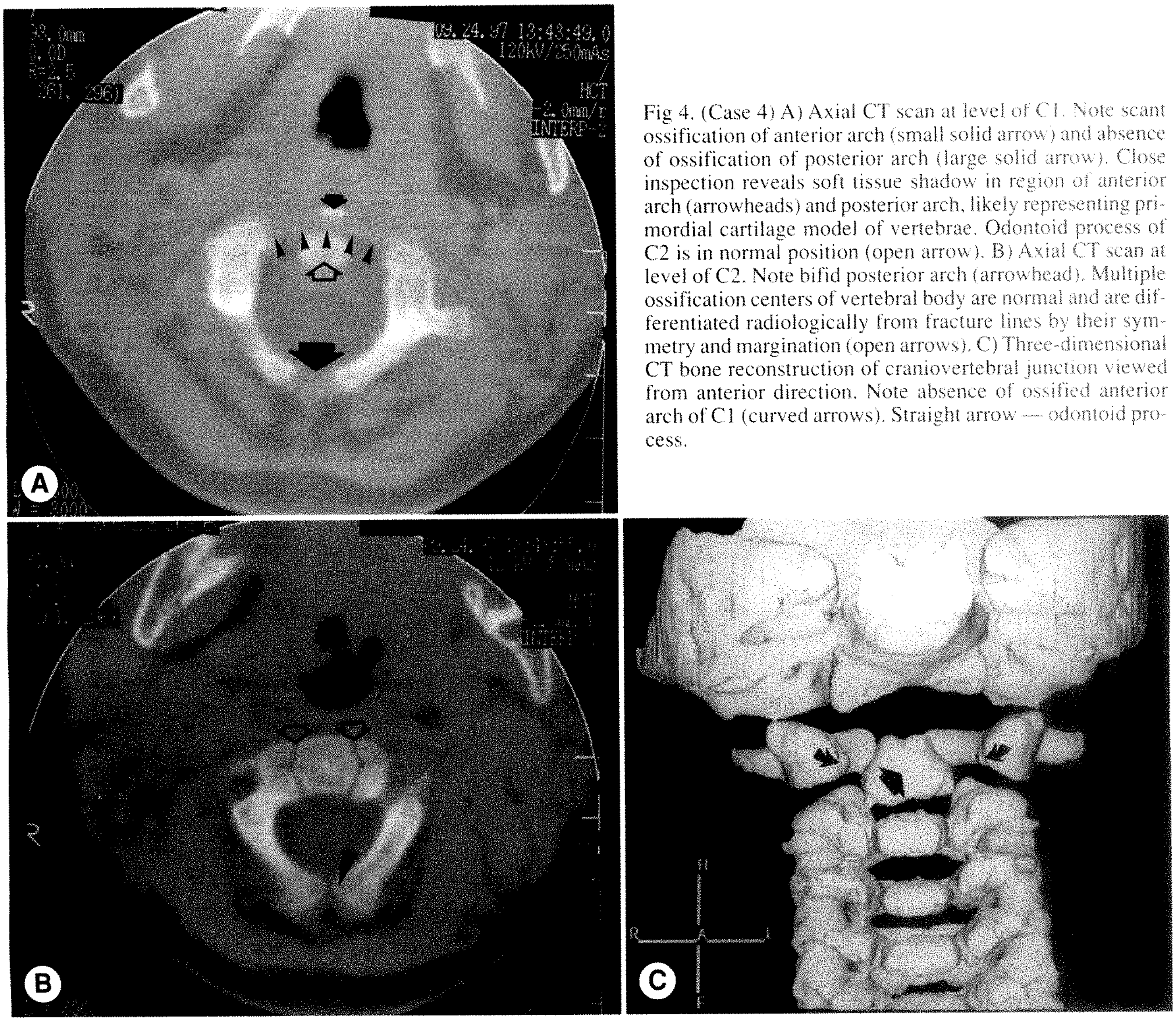

Fig 4. (Case 4) A) Axial CT scan at level of C1. Note scant ossification of anterior arch (small solid arrow) and absence of ossification of posterior arch (large solid arrow). Close inspection reveals soft tissue shadow in region of anterior arch (arrowheads) and posterior arch, likely representing primordial cartilage model of vertebrae. Odontoid process of C2 is in normal position (open arrow). B) Axial CT scan at level of C2. Note bifid posterior arch (arrowhead). Multiple ossification centers of vertebral body are normal and are differentiated radiologically from fracture lines by their symmetry and margination (open arrows). C) Three-dimensional CT bone reconstruction of craniovertebral junction viewed from anterior direction. Note absence of ossified anterior arch of C1 (curved arrows). Straight arrow — odontoid process.

inspiratory-expiratory positive airway pressure and referred to our institution for possible decannulation. Severe suprastomal collapse was noted, and the patient was decannulated following single-stage laryngotracheal reconstruction.

By 7 years of age, severe cervicothoracic kyphoscoliosis had destabilized both the cervical and thoracic vertebrae. A posterior fusion of C6 through T12 was performed with anterior release and a rib strut graft from C6 to T4. The patient failed multiple attempts at extubation following the surgery, and a tracheotomy tube was replaced for prolonged ventilatory support. Seven months later, the patient was decannulated following revision single-stage laryngotracheal reconstruction.

DISCUSSION

Knowledge of cervical vertebral embryology is instrumental in understanding the pathogenesis of developmental anomalies. The neural tube forms early in embryological development. At 16 to 18 days of gestation, mesodermal cells condense in the midline to form the notochordal process. During weeks 2 to 4, the notochordal process flattens and degenerates to form the notochordal plate, which in turn invaginates to form the notochord. During this same time period, the overlying neural plate thickens and also invaginates, forming the neural groove and folds. By the fourth week, the neural folds fuse to form the neural tube. Vertebral formation begins simultaneously, when sclerotomal mesoderm from the somites migrates toward the notochord.[2] In a phenomenon called *Neugliederung* (resegmentation), the caudal half of each sclerotome thickens and fuses with the cephalad half of the next sclerotome. Each vertebra is thus composed of 2 different halves of adjacent sclerotomes. Subsequently, the mesenchymal cells of the primordial vertebra grow ventrally to form the vertebral body, dorsally to form the vertebral arch, and laterally to form the transverse processes and ribs.

Chondrification of the primitive vertebral column begins during week 6 of gestation. The vertebral arch does not fuse until chondrification is completed at week 12. Before chondrification is complete, ossification starts during week 8 in ossification centers of the vertebrae in which cartilage is already formed. Generally, ossification should be complete enough that the anterior and posterior arches of the vertebrae are evident radiographically at birth. Fusion of the spinous, articular, and transverse processes is actually not complete until the seventh year of life.

Development of both a notochord and a neural tube is necessary for complete cartilaginous induction of the vertebrae from the sclerotomal mesoderm. Experimental removal of the neural tube in animals causes failure of vertebral arch formation, and removal of the notochord results in agenesis of the vertebral body.[3]

Much of the development of the major head and neck structures also occurs between 4 and 12 weeks of gestation.[4-7] The temporal overlap and spatial association of the developing systems helps to explain why anomalies of the vertebral column should be considered in the presence of other anomalies of the head and neck.

Insults to the maturing embryo may occur in the form of intrauterine trauma, vascular insufficiency, or systemic teratogens, including ethyl alcohol, anticonvulsants, vitamin A congeners, folate antagonists, and coumadin.[1] Inherited abnormalities can arise as the result of germ-line mutations involving molecular pathways critical for mesenchymal condensation, resegmentation, and/or cartilaginous induction.[8-11] For example, the mammalian homeotic gene (Hox) complex includes a family of 38 genes clustered into 4 linkage groups on 4 different chromosomes.[12] These linkage groups (termed HoxA, HoxB, HoxC, and HoxD) share common DNA-binding sequence motifs known as the homeo domain. Within each Hox linkage group, each gene can be grouped with at least 2 other genes from different clusters on the basis of DNA sequence homology. Expression is sequentially regulated on the anteroposterior axis of the embryo, leading to the formation of 13 paired groups within the Hox gene family.[8] Disruption of specific Hox genes alters vertebral development in mice, giving rise to phenotypes characterized by the formation of C2 anterior arches, cervical ribs, and shoulder shifting.[9] These changes are similar to those of the Sprengel deformity and Klippel-Feil anomaly seen in humans.

Our experience suggests that most vertebral anomalies are asymptomatic. However, the apnea episodes experienced in case 1 and the traumatic quadriparesis experienced in case 3 illustrate the potential morbidity that can occur if diagnosis and treatment are delayed. Neurologic symptoms arise from compression of the brain stem, cranial roots and nerves, spinal cord, or vertebral arteries. Apnea, dysphagia, sensorineural hearing loss, nystagmus, internuclear ophthalmoplegia, suboccipital pain, syncope, vertigo, attacks of altered consciousness, transient loss of visual fields, and acute episodes of quadriplegia can result.[13-15] Torticollis is the most common muscular presentation of craniovertebral junction anomalies. Most cases of torticollis in infants are due to fibromatosis colli (a benign condition thought to arise from intrauterine positioning), but if classic features of torticollis are not present, the possibility of cervical spine abnormalities must be considered.

The association of vertebral anomalies in patients with craniofacial syndromes warrants vertebral imaging in select cases. Imaging studies are routinely obtained in patients with trisomy 21 due to the increased risk of atlantoaxial subluxation.[16,17] Flexion and extension films should be obtained between 2 and 3 years of age and should be repeated in the early teenage and adult years, with the onset of neurologic changes (case 3), and before participating in sports, especially the Special Olympics.[16] Narrowing of the spinal canal with cord compromise due to movement of C1 in relation to C2 requires surgical stabilization.[17] However, normal findings on imaging studies do not eliminate the risk of atlantoaxial subluxation upon relaxation of the cervical muscles under anesthesia, and all patients with trisomy 21 should be managed as if they have cervical spine instability when under general anesthesia.[15]

Vertebral anomalies are seen relatively commonly with Goldenhar's syndrome (case 2). On the basis of this association, it is our recommendation that these patients have cervical spine imaging at the time of diagnosis or during early childhood. Had our patient presented before 14 years of age, surgical correction would have been considered to maintain vertebral height, prevent progressive scoliosis, and prevent narrowing of the spinal canal. We would also recommend craniocervical evaluation in patients with Apert syndrome, Crouzon syndrome, or any nonsyndromic craniosynostosis.

The craniovertebral junction is the most commonly affected site of cervical vertebral anomalies and comprises the occipital bone surrounding the foramen magnum, the atlas (C1), and the axis (C2). It is not uncommon for pediatric patients with head and neck anomalies to have imaging studies performed prior

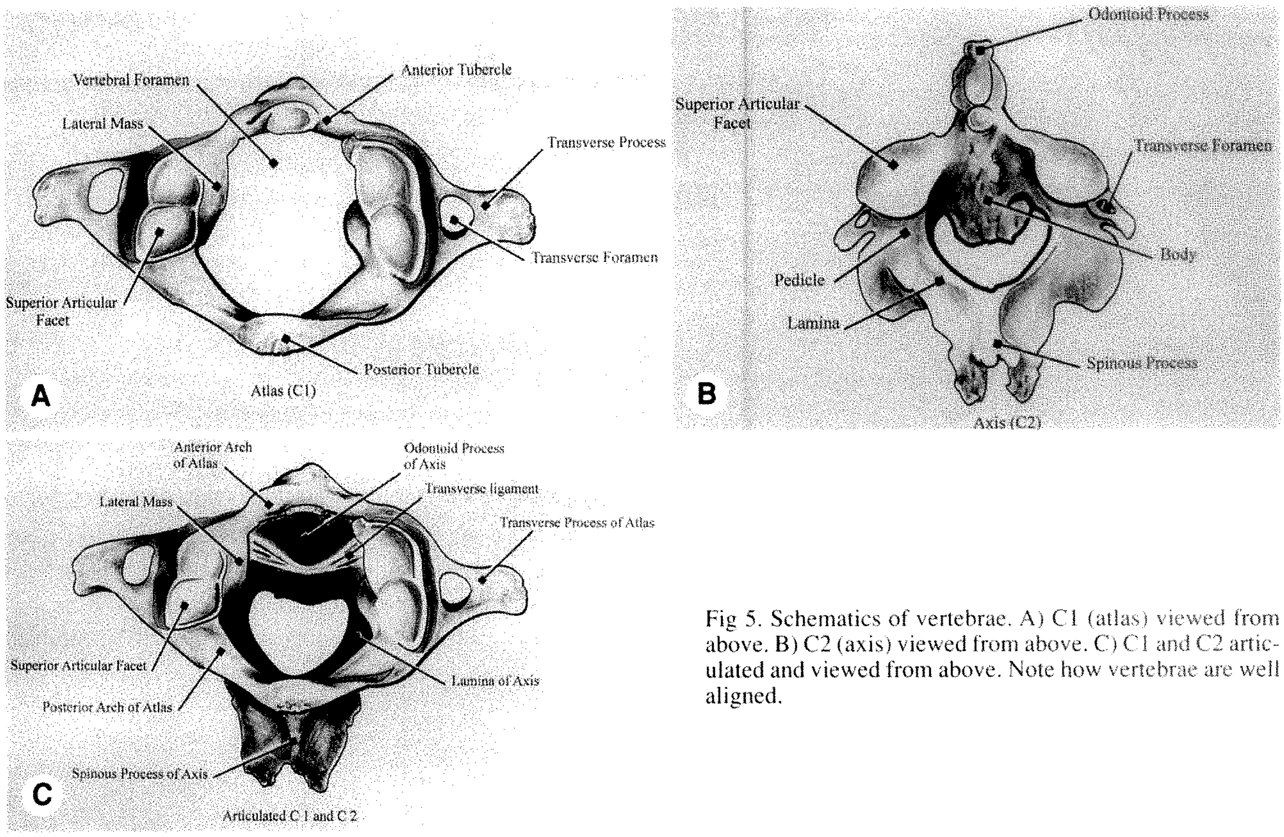

Fig 5. Schematics of vertebrae. A) C1 (atlas) viewed from above. B) C2 (axis) viewed from above. C) C1 and C2 articulated and viewed from above. Note how vertebrae are well aligned.

to presenting to an otolaryngologist, so a review of basic radiologic anatomy of the craniovertebral junction is presented.

Most cervical vertebral anomalies can be detected by plain radiography if lateral, anteroposterior, open mouth, and oblique views are assessed, but obtaining this series of films in infants and toddlers is difficult.[13] Cervical flexion and extension films provide information about the biomechanics and stability of the cervical vertebrae, but are also not easily obtained in young patients. Computed tomography provides excellent resolution of the vertebrae and has become the imaging study of choice. As noted in Case Reports, the alignment of the vertebrae is seen even more clearly with 3-dimensional reconstruction of the images. Magnetic resonance imaging may complement CT scan results by assessing compression of the brain stem and spinal cord.

In reviewing imaging studies, the presence, configuration, and alignment of each component of the craniovertebral junction must be assessed. The foramen magnum adjoins the cranial cavity and the vertebral canal and is housed by the occipital bone. The basiocciput (clivus) forms the anterior margin of the foramen magnum, and the ex-occiput, the posterior (Fig 5A). The occipital condyles articulate with the atlas and are located anterolaterally on the caudal aspect of the occipital bone.

The atlas is an irregularly ring-shaped vertebra that serves as a "washer" between the cranium and the cervical spine. The anterior and posterior arches of the atlas are joined by large lateral masses that bear much of the weight of the skull and articulate with the occipital condyles (Fig 5A). The transverse processes arise from the lateral masses and house the transverse foramen through which the vertebral arteries course. From the medial aspect of each lateral mass projects the transverse ligament that divides the spinal canal into an anterior compartment housing the odontoid process and a posterior compartment housing the spinal cord, meninges, and blood vessels. The atlas is easily differentiated radiologically from the remaining cervical vertebrae, as it lacks both a body and a spinous process.

Like the remaining cervical vertebrae, the anterior aspect of the axis (C2) is defined by a broad vertebral body (Fig 5B). The vertebral body is not completely ossified at birth, but the junctions of its several ossification centers should not be confused with fracture lines, because of their symmetric appearance and their margination. The odontoid process (dens) projects 1.0 to 1.5 cm above the vertebral body, providing the axis with its most distinguishing feature. Anteriorly, the odontoid process articulates with the posterior border of the anterior arch of the atlas; posteriorly, it abuts against the transverse ligament (Fig

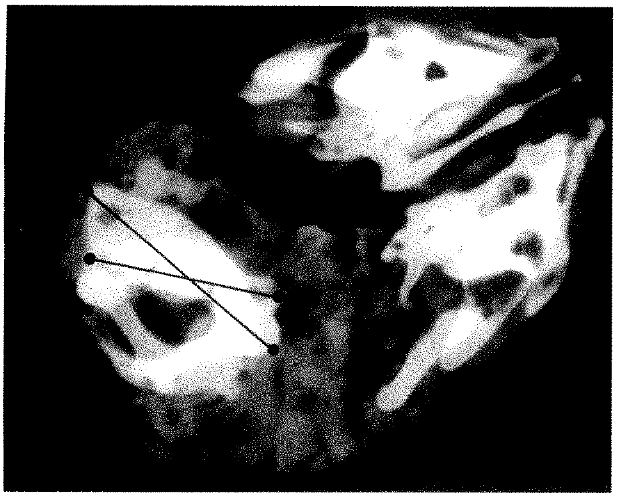

Fig 6. Superimposed axial CT scans at level of C1 and C2, demonstrating rotatory subluxation. Note that transverse foramina of adjacent vertebrae are rotated out of alignment by 22° (lines).

5C). The laminae forming the posterior aspect of the vertebral foramen are joined to the vertebral body by pedicles from which arise the transverse processes.

In addition to the configuration of each component of the craniovertebral junction, the alignment between adjacent structures is important to assess. Rotation of the vertebrae is best assessed by superimposing axial sections through the occipital condyles and C1 and C2 with the films in a neutral position (Fig 6).

Vertical alignment is best assessed on coronal or sagittal CT reconstructions. Vertical migration of the odontoid process above the level of the foramen magnum in the coronal or sagittal plane indicates basilar invagination. This phenomenon may occur if the transverse and cruciate ligaments are lax, as is thought to occur in children with Down syndrome. Basilar invagination may also be evident on axial CT images, as illustrated in case 3, in which the odontoid process of C2 and the posterior arch of C1 severely narrowed the foramen magnum and compressed the medulla and spinal cord (Fig 3B). The patient's symptoms of cyanosis and acute quadriparesis were likely precipitated by neck flexion, which allowed the superiorly positioned odontoid process to further compress the cervicomedullary region.

Horizontal alignment is assessed by drawing a line along the lateral edge of the occipital condyle and the lateral borders of C1 and C2 on frontal reconstructed CT images. If such a line cannot be drawn smoothly, then lateral override may exist.

Patients in whom vertebral anomalies are evident, or even suspected, should be referred to a neurosurgeon for evaluation and counseling on activity precautions. Unstable anomalies may require immobilization with a cervical collar or halo reduction and fusion. As noted in case 2, even stable anomalies may require surgical intervention to prevent progressive scoliosis and/or compensatory kyphosis. Direct laryngoscopy and endotracheal intubation often are exceedingly difficult in these patients; however, intubation over a flexible fiberoptic bronchoscope or management with a laryngeal mask airway may obviate the difficulties.

CONCLUSIONS

1. Cervical spine anomalies often coexist with syndromic anomalies of the head and neck and should be detected by radiographic imaging as early as possible to optimize management and reduce the risk of neurologic injury.

2. Insults to the developing embryo between weeks 4 and 12 of gestation may affect the axial skeleton and the major head and neck structures. Such insults include vascular insufficiency, compressive forces, intrauterine trauma, systemic teratogens, and germline mutations.

3. Vertebral anomalies may be difficult to distinguish from fibromatosis colli in infants who present with torticollis. Craniocervical evaluation is recommended for infants who do not have classic congenital torticollis.

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REFERENCES

1. Gorlin RJ, Cohen MM, Levin LS, eds. Syndromes of the head and neck. 3rd ed. New York, NY: Oxford University Press, 1990.

2. Lonstein JE. Spine embryology. In: Winter RB, Lonstein JE, Leonard AS, eds. Congenital deformities of the spine. New York, NY: Thieme-Stratton, 1983:1-10.

3. Watterson RL, Fowler I, Fowler BJ. The role of the neural tube and notochord in development of the axial skeleton of the chick. Am J Anat 1954;95:337-99.

4. Kenna MA. Embryology and developmental anatomy of the ear. In: Bluestone CD, Stool SE, Kenna MA, eds. Pediatric otolaryngology. Vol 1. Philadelphia, Pa: WB Saunders, 1996: 113-26.

5. Anson BJ, Davies J, Duckert LG. Embryology of the ear. In: Paparella MM, Shumrick DA, Gluckman JL, Meyerhoff WL, eds. Otolaryngology. 3rd ed. Vol 1. Philadelphia, Pa: WB Saunders, 1991:3-21.

6. Tomaski S. Embryology and anatomy of the mouth, pharynx and esophagus. In: Bluestone CD, Stool SE, Kenna MA, eds. Pediatric otolaryngology. Vol 2. Philadelphia, Pa: WB Saun-

ders, 1996:918-38.

7. Isaacson G. Developmental anatomy and physiology of the larynx, trachea, and esophagus. In: Bluestone CD, Stool SE, Kenna MA, eds. Pediatric otolaryngology. Vol 2. Philadelphia, Pa: WB Saunders, 1996:1202-11.

8. Scott MP. Vertebrate homeobox gene nomenclature. Cell 1992;71:551-3.

9. Rancourt DE, Tsuzuki T, Capecchi MR. Genetic interaction between hoxb-5 and hoxb-6 is revealed by nonallelic noncomplementation. Genes Devel 1995;9:108-22.

10. Wilting J, Ebensperger C, Muller TS, Koseki H, Wallin J, Christ B. Pax-1 in the development of the cervico-occipital transitional zone. Anat Embryol (Berl) 1995;192:221-7.

11. Baldwin CT, Hoth CF, Macina RA, Milunsky A. Mutations in PAX3 that cause Waardenburg syndrome type I: ten new mutations and review of the literature. Am J Med Genet 1995;58:115-22.

12. Duboule D, Dolle P. The structural and functional organization of the murine Hox gene family resembles that of *Drosophila* homeotic genes. EMBO J 1989;8:1497-505.

13. Menezes AH. Congenital and acquired abnormalities of the craniovertebral junction. In: Youmans J, ed. Neurological surgery. 4th ed. Vol 2. Philadelphia, Pa: WB Saunders, 1995: 1035-89.

14. Fremion AS, Garg BP, Kalsheck J. Apnea as the sole manifestation of cord compression in achondroplasia. J Pediatr 1984; 104:398-401.

15. Menezes AH. Complications of surgery at the craniovertebral junction. Avoidance and management. Pediatr Neurosurg 1992;17:254-66.

16. Kraus EM. Down syndrome and the otolaryngologist: clinical characteristics and recommendations for management. Curr Concepts Otolaryngol 1992;5:3-16.

17. Menezes AH, Ryken TC. Craniovertebral abnormalities in Down syndrome. Pediatr Neurosurg 1992;18:24-33.

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IMMORTALIZATION OF CHINCHILLA MIDDLE EAR EPITHELIAL CELLS BY ADENOVIRUS 12–SIMIAN VIRUS 40 HYBRID VIRUS

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In order to study the cellular and molecular mechanisms of the pathogenesis of otitis media, a chinchilla middle ear epithelial cell line (CMEE-1) with differentiated cell characteristics was established by infection of a primary culture with the adenovirus 12–simian virus 40 (Ad12-SV40) hybrid. This cell line has been in continuous culture for 42 passages, whereas the parent cells underwent senescence and died at the 8th passage. The cell line also retains epithelial morphology and expresses cytokeratin polypeptides 4, 7, and 18, characteristic markers for epithelia. In Western blots of cell proteins, bands at 94 and 53 kd were labeled after binding antibodies against SV40 large T antigen and p53, respectively. Karyotype analysis showed that the cell line is derived from chinchilla epithelial cells. These findings confirm that the cell line is a chinchilla epithelial cell immortalized by the hybrid virus.

KEY WORDS — adenovirus 12–simian virus 40, cell culture, cell line, chinchilla middle ear epithelial cell, immortalization.

INTRODUCTION

The chinchilla has been widely used as an experimental model for otitis media because of its large bulla and its susceptibility to pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenzae*, which cause otitis media in humans.[1-3] The chinchilla middle ear epithelial cell line (CMEE-1) is an important tool in studying the role of middle ear epithelial cells in the pathogenesis of otitis media. Studies involving bacterial–host cell interaction and the expression of bacterial receptors on middle ear epithelial cells, for example, will be greatly facilitated by the CMEE-1 cell line.

Since van Blitterswijk et al[4] first reported a successful cultivation of rat middle ear epithelial cells, a number of investigators have primarily or serially cultured chinchilla middle ear epithelial cells.[5-8] Although they were successful in establishing primary or serial cultures of chinchilla middle ear epithelial cells, the cultures were of limited utility because of the short life span and limited proliferative ability of the chinchilla middle ear epithelial cells. Hence, it is impossible to perform pathogenetic studies using large numbers of homogeneous cell populations without sacrificing several animals. Immortalization of middle ear epithelial cells from the Mongolian gerbil and rat has been reported.[9-11] The present study provides the first evidence of immortalization of chinchilla middle ear epithelial cells.

MATERIALS AND METHODS

Animals. A total of 18 healthy chinchillas (*Chinchilla laniger*, male, body weight 300 to 400 g) without middle ear disease were used.

Culture Medium. The culture medium was the same as previously described by Nakamura et al[5,6] with minor modifications. Our culture medium consisted of a mixture of 3 parts of Dulbecco's modified Eagle medium (Gibco) and 1 part of nutrient mixture F12 (Ham's F12, Gibco) supplemented with 10% fetal bovine serum (Gibco), hydrocortisone (0.4 μg/mL; Sigma), isoproterenol (10^{-6} mol/L; Sigma), and antibiotic-antimycotic (1:100 dilution; Gibco).

Primary Culture. The chinchillas were placed under general anesthesia with ketamine hydrochloride at a dose of 40 mg/kg, intramuscularly (Fort Dodge Laboratories, Inc). After the chinchilla bullae were removed and opened, the middle ear mucosa was separated from the inferior bulla near the tympanic orifice of the eustachian tube. To minimize bacterial contamination, the mucosa was immersed in normal saline with antibiotics (penicillin 100 U/mL, streptomycin 100 U/mL, amphotericin 0.25 μg/mL) for 1 hour at 4°C. Under sterile conditions, the mucosa was chopped into very fine pieces and then placed

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From the Laboratory of Cellular Biology, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Rockville (Jin, Gu, Lim), and the Laboratory of Biochemical Physiology, National Cancer Institute, National Institutes of Health, Frederick (Rhim), Maryland, and the Department of Cell and Molecular Biology, House Ear Institute, Los Angeles, California (Lim). This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the National Institutes of Health.

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in 35-mm tissue culture dishes coated with collagen type 1 (Biocoat). A total of 30 such dishes were incubated in a carbon dioxide incubator at 37°C. After 24 hours, 2 mL of culture medium was added to each dish and incubation was continued. After 3 or 4 days, when outgrowth was observed from the explant, the culture medium was changed twice a week thereafter for continuous growth. By the 8th day in culture, all explants of the middle ear mucosa were removed. If fibroblasts were found on the outgrowth in some dishes, they were physically removed with a scalpel under the microscope. Contaminated culture dishes were discarded.

Infection of Ad12-SV40 Hybrid Virus. On day 16 in primary culture, 8 of the primary culture dishes that had achieved 80% confluence were selected for viral infection. These primary cells were infected by adding 0.25 mL of a 1:10 dilution of adenovirus 12–simian virus 40 (Ad12-SV40) hybrid virus in each culture dish and incubated for 3 days. The medium was then removed and 2 mL of fresh medium was added to the cells. The infected cells were allowed to grow to 100% confluence and subcultured. As a control, 5 of the primary culture dishes were subjected to continuous culture. Presence of infectious virus in the culture medium of the virus-infected cells from passages 8 to 10 was checked 3 times by inoculating the culture supernatant to Vero cell cultures as described.[12] After 21 days, if the Vero cells died or appeared pathological, the culture was considered to be a virus producer.

Proliferation and Doubling Time. A growth curve was plotted and the doubling time was calculated in order to determine the proliferative ability of the CMEE-1 cells; 1×10^5 of the CMEE-1 cells (passage 18) and the parent control cells (passage 3) were seeded in a 60-mm petri dish. The number of cells in 3 of the CMEE-1 dishes and 2 of the uninfected parent control dishes were counted with a hemocytometer for each time point, and the average numbers for the cell line and parent cells were calculated. The growth assay was performed at day 8. Depending on the average numbers, 2 growth curves were made and the number of generations and doubling time in the assay were calculated for the cell line and the control cells. The exponential was represented as $N = N0 2^x$ or $\log 10N = \log 10N0 + x\log 102$, where N is the final cell number, N0 is the initial cell number, and x is the number of generations of exponential growth. The doubling time during exponential growth was calculated as follows: Doubling time = Total time elapsed/Number of generations. The saturation density of CMEE-1 cells and parent control cells in 60-mm petri dishes was determined by continuous cell count followed by a growth assay over a period of 2 days.

Morphology. For light microscopy, the immortalized cells and the parent control cells were seeded in 60-mm petri dishes or 75-cm^2 flasks and were examined and photographed with a phase contrast microscope (Zeiss Axiovert 25). For scanning electron microscopy (SEM),[5] the cells cultured on the glass coverslips were rinsed with phosphate-buffered saline (PBS; pH 7.2) and fixed with 2.5% glutaraldehyde in 100-mmol/L cacodylate buffer overnight. After washing with cacodylate buffer, the cells were immersed in a saturated solution of thiocarbohydrazide for 30 minutes and then in 1% osmium tetroxide for 1 hour. After washing with distilled water, the last 2 steps were repeated. Following graded dehydration with ethanol, the specimens were critical-point dried with carbon dioxide and sputter-coated with gold, and examined by SEM (Hitachi, S-3200N). For transmission electron microscopy (TEM),[5] the cells cultured on Thermanox coverslips (Nunc, Inc) were fixed with 3% glutaraldehyde in 100-mmol/L cacodylate buffer at 4°C for 3 hours before postfixation in 1% osmium tetroxide at 4°C for 4 hours. The specimens were dehydrated in a graded series of ethanol, embedded in Spurr's medium (Electron Microscopy Sciences, Fort Washington, Pa), and then cured at 60°C for 12 hours. Thin sections were cut perpendicular to the coverslip, mounted onto grids, stained with uranyl acetate and lead citrate, and then examined by TEM (Zeiss, EM 902A).

Immunocytochemistry. Indirect immunofluorescence was used to detect SV40 large T antigen, p53, cytokeratin, and vimentin staining. Cells cultured on collagen type 1–coated coverslips were fixed with prechilled methanol (–20°C) for 20 minutes, permeabilized with acetone (4°C) for 2 minutes, dried, and rinsed with PBS. These cells were blocked with 10% normal goat serum for 30 minutes at 37°C and then incubated with primary antibodies for 1 hour at 37°C. After washing, the cells were incubated with goat anti-mouse IgG conjugated with fluorescein isothiocyanate isomer I (FITC; Sigma) for 1 hour at 37°C. After washing, the coverslips were mounted on glass slides with glycerin mounting gel and observed with a light microscope equipped with incident-light fluorescence (Zeiss, Axiovert 135).

As primary antibodies, 13 types of cytokeratin antibodies (Sigma) were used in cytokeratin staining to characterize cell types as shown in Table 1.[13-26] These cytokeratin antibodies recognize cytokeratin polypeptides 1, 4-8, 10, 11, 13, and 15-19. Monoclonal anti-vimentin (clone No. V9; Sigma), which labels vimentin in fibroblast cells, was employed to rule out fibroblast contamination.[27] Mouse monoclo-

TABLE 1. ANTI-CYTOKERATIN ANTIBODIES

Antibody	*Clone No.*	*Polypeptide Specificity*	*Cell Specificity*	*References*
Anti-cytokeratin peptide 14	CK-B1	14	Myoepithelial cells of gland, basal layer of squamous epithelium	13
Anti-cytokeratin peptide 17	CK-E3	17	Myoepithelial cells of gland	14, 15
Anti-cytokeratin 4.62	K4.62	19	Simple, transitional, and metaplastic epithelia	16, 17
Anti-cytokeratin peptide 8	M20	8	Simple and complex epithelia	14, 18
Anti–pan-cytokeratin	PCK-26	1, 5, 6, 8	Simple and pseudostratified epithelia, cornifying and noncornifying squamous epithelia	19, 20
Anti-cytokeratin 8.12	K8.12	13, 15, 16	Basal layer of pseudostratified epithelium, nonkeratinized squamous epithelium, transitional epithelium	17, 21
Anti-cytokeratin 8.60	K8.60	10, 11	Suprabasal layer of epidermis, individual cells in squamous epithelium	16
Anti-cytokeratin peptide 4	6B10	4	Noncornifying squamous and transitional epithelia	22, 23
Anti-cytokeratin peptide 7	LDS-68	7	Simple epithelium	17, 24
Anti-cytokeratin peptide 18	CY-90	18	Simple epithelium	20, 25
Anti–pan-cytokeratin	C-11	4, 5, 6, 8, 10, 13, 18	Most simple epithelia, cornifying and noncornifying squamous epithelia	20, 26
Anti-cytokeratin 8.13	K8.13	1, 5, 6, 7, 8, 10, 11, 18	Epithelia	16, 17
Anti–pan-cytokeratin	Mixture of clones	1, 4, 5, 6, 8, 10, 13, 18, 19	Simple and pseudostratified epithelia, cornifying and noncornifying squamous epithelia	14, 16-20, 26

nal antibody against SV40 large T antigen (SV40 TAg; Ab-2) and mouse monoclonal antibody against p53 (P53; Ab-1; Oncogene Research Products, Cambridge, Mass) were used to stain the SV40 large T antigen and p53. The specificity of these monoclonal antibodies against SV40 large T antigen and p53 has previously been established by the vendor. Vero cells were used as a negative control in the cytokeratin staining and as a positive control in the vimentin staining. The parent cells (non–virus-infected) were used as a negative control.

Colony Formation in Soft Agar. The CMEE-1 and parent control cell suspensions (5×10^4 cells per milliliter) in 2 mL of 0.36% Noble agar with culture medium were overlaid onto a 60-mm petri dish containing 5 mL of 0.96% base agar. We used KB cells as a positive control. Viable colonies of each kind of cells were scored after 21 days.

Western Blot. The Western blot procedure of Towbin et al[28] was used with modifications. Briefly, confluent cultures of the cells were washed with PBS (pH 7.2) and suspended in buffer containing 100-mmol/L sodium chloride and 10-mmol/L Tris (pH 7.6) with protease inhibitor cocktail and pepstatin (Boehringer Mannheim, Inc) at 4°C. One volume of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer was added, and the mixture was boiled for 10 minutes before running on a 7.2% gel. The proteins were transferred from gels onto nitrocellulose membranes at 250 mV for 3 to 4 hours in Bio-Rad transfer buffer (pH 7.5). The membranes were blocked with 3% skim milk and then incubated with mouse monoclonal antibodies against SV40 large T antigen or p53 for 2 hours. Alkaline phosphatase–conjugated anti-mouse IgG was used as a secondary antibody. The membranes were developed in 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt and nitroblue tetrazolium chloride substrate solution (Sigma) at room temperature to permit visualization of the bands.

Chromosomal Analysis. Karyotypic analysis[29] was carried out by Dr Bharati Hukku, Children's Hospital of Michigan, Detroit, Michigan.

RESULTS

Primary Culture. A successful outgrowth of the chinchilla middle ear epithelial cells was obtained in primary culture. By the 3rd day after explantation, it was demonstrated that 236 of the 270 explants (87.4%) had attached to the surface of petri dishes and that an outgrowth of the epithelial cells was observed in most explants. On the 8th day after explantation, 221 of the 270 explants (82%) demonstrated outgrowth. Thirteen of the 30 primary cultures were selected for virus infection (8) or as controls (5) on the basis of healthy growth and lack of fibroblast contamination.

Immortalization. Seven weeks after virus infection, 1 of the 8 virus-infected cultures displayed higher growth than the others. These rapidly growing cells

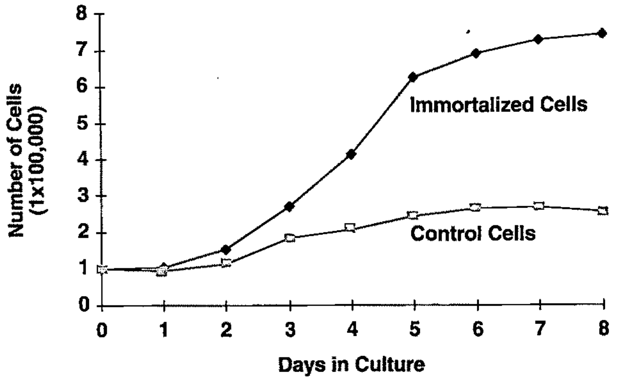

Fig 1. Growth curves of CMEE-1 cells and parent control cells. Immortalized CMEE-1 cells (passage 18) grow faster than parent control (passage 3), noninfected cells.

reached confluence within 1 week when subcultured at a 1:2 ratio, while the parent control cells reached confluence within 2 weeks. The high-growth chinchilla middle ear epithelial cells (designated as CMEE-1) maintained steady growth potential and continued to proliferate for up to 42 passages over 10 months. However, the parent controls underwent senescence and died within 8 passages (4 months). Vero cell cultures inoculated with a supernatant from the CMEE-1 cells showed no evidence of cytopathic effects.

Proliferation and Doubling Time. A distinct lag phase and a distinct log phase were observed for the growth curve of the CMEE-1 cells. The lag phase showed negligible growth, but the log phase showed a strong exponential growth. The parent control cells displayed negative growth at lag phase and slower growth at log phase. The immortalized cells showed a weak but sustained proliferative ability after log phase over a period equal to the plateau phase, while the parent control cells displayed a short negative growth period at the end of their growth curve (Fig 1). At the 8th day, the generation number of the immortalized cells was 2.89, and the average doubling time was 66.43 hours, while the generation number of the parent control cells was 1.33, and the doubling time was 144.36 hours (Table 2). In log phase, the immortalized cells doubled 2.63 times, and the doubling time was 45.62 hours; in contrast, the parent control cells doubled 1.38 times, and the doubling time was 86.59 hours. The saturation density of the CMEE-1 cells was found to be 7.13×10^5, while the parent control cells had a saturation density of 2.67×10^5 cells.

Morphology. When trypsinized, plated, and visualized after 5 hours, the CMEE-1 cells appeared spherical but flattened out with ruffled edges. Several filopodia extended from the ruffled edges. The epithelial cells established cell-to-cell contact by some filopodial projection, then became a confluent monolayer. Tight junctions appeared after the cells were confluent, and then the typical cobblestone-like morphology was observed on the cell monolayer (Fig 2A,B). A dome formation, which was frequently observed in primary cultures of chinchilla middle ear epithelial cells,[2] was rarely observed in CMEE-1 cells.

TABLE 2. CHARACTERISTICS OF CMEE-1 AND PARENT CONTROL CELLS

	CMEE-1	*Parent Control Cell*
Life span	>42 Passages	<8 Passages
Colony formation in soft agar	–	–
Saturation density (60-mm dish)	7.13×10^5	2.67×10^5
Average doubling time (h)	66.43	144.36
Number of generations (8 d)	2.89	1.33
Cytokeratin polypeptides	4, 7, 18	4, 7, 18
Vimentin	–	–
SV40 large T antigen	+	–
p53	+	–

Under a light microscope, the CMEE-1 cell shapes were not uniform at the lower passages. With increasing passages, the cell shapes gradually became uniform and smaller in size with a polygonal shape, a distinct cell junction, and a prominent nucleus. However, the parent cells retained similar epithelial shapes and size with increasing passages before the cells underwent senescence. The parent cells were larger than the CMEE-1 cells. The CMEE-1 cells continued to divide actively, as demonstrated by the presence of mitotic figures (Fig 2A). After about the 10th passage, most, if not all, CMEE-1 cells in monolayer became uniform.

With increasing passages (up to about 30 passages), some of the cells changed their morphology. A number of elongated fibroblast-like cells without distinct cell boundaries appeared in the cultures, and some cells showed a few abnormal filopodia. In order to rule out the possibility of fibroblast contamination, the high-passage cells were stained with cytokeratin antibody. These morphologically changed cells, including the abnormal filopodia, were 100% positive in expression of epithelial cell cytokeratin (clone No. C-11). The ciliated cells that had been observed in the early stage of primary culture were not observed in the advanced passages of the parent cells and were never observed in the CMEE-1 cell line.

Upon SEM examination, numerous microvilli of different lengths (0.1 to 3 μm) were observed in both CMEE-1 cells and parent control cells. The density of microvilli varied from cell to cell, and some cells

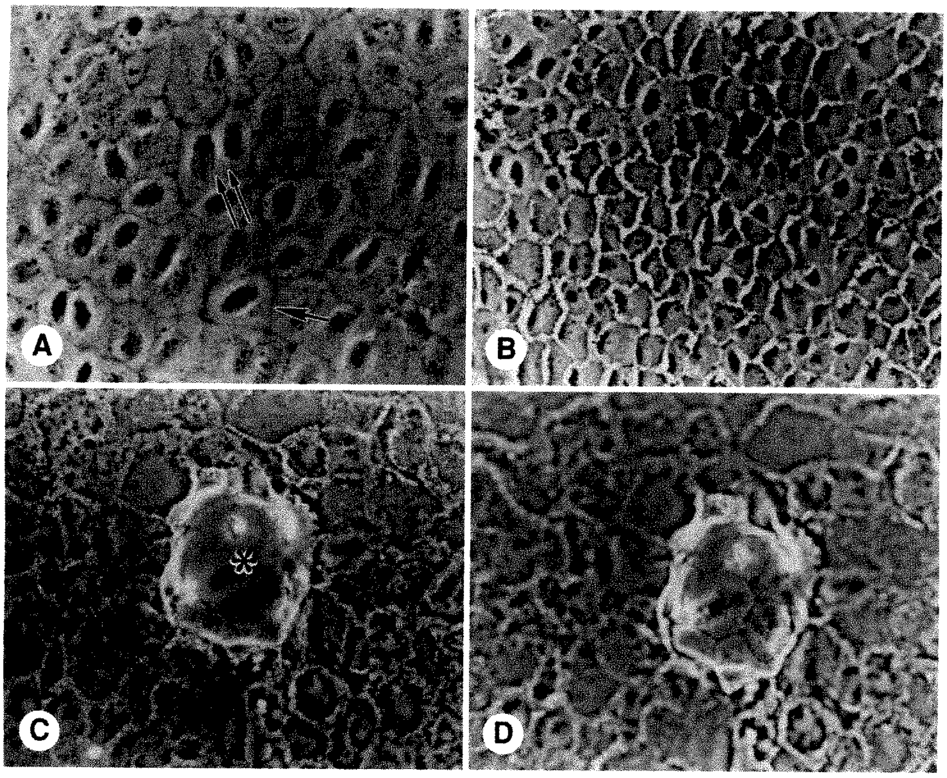

Fig 2. Phase-contrast light microscopic photograph (original ×200). A) Sixteenth-passage CMEE-1 cells after 9 days in culture. Polygonal cell shape with tight junction (single arrow) and large cells with double nuclei (double arrows) that are interpreted to be dividing cells are shown. B) Sixteenth-passage CMEE-1 cells after 12 days in culture. Cobblestone-like appearance that is result of tight junction formation is shown. C) Fifteenth-passage CMEE-1 cells. Dome formation after 14 days in culture (asterisk). D) Top of dome showing polygonal cell shape and tight junction.

showed an uneven distribution of microvilli. For example, in Fig 3B, the distribution of microvilli in 1 part of the cell is sparse, while the rest of the cell shows a high density of microvilli. Extremely long microvilli often occurred in clusters intermixed with short microvilli (Fig 3A), and such long microvilli were never observed in primary culture. No ciliated cells were observed.

The TEM observation of uninfected parent control cells showed a monolayer of epithelial cells with microvilli, clear vesicles, tight junctions, numerous mitochondria, and a well-developed Golgi apparatus and rough endoplasmic reticulum. Some cells contained large amounts of osmiophilic particles. Their TEM appearance resembled that of glycogen. In some instances, cultures of 2 or 3 cell layers were formed. The CMEE-1 cells showed distinct tight junctions between cells. Most of the CMEE-1 cells showed numerous microvilli of different lengths and shapes. Some of the microvilli were branched. Sometimes, the CMEE-1 cells grew in 2 or 3 thin layers (Fig 4).

Immunocytochemistry. The cytokeratin staining patterns of the parent control cells and the immortalized cells were identical. Both kinds of cells were

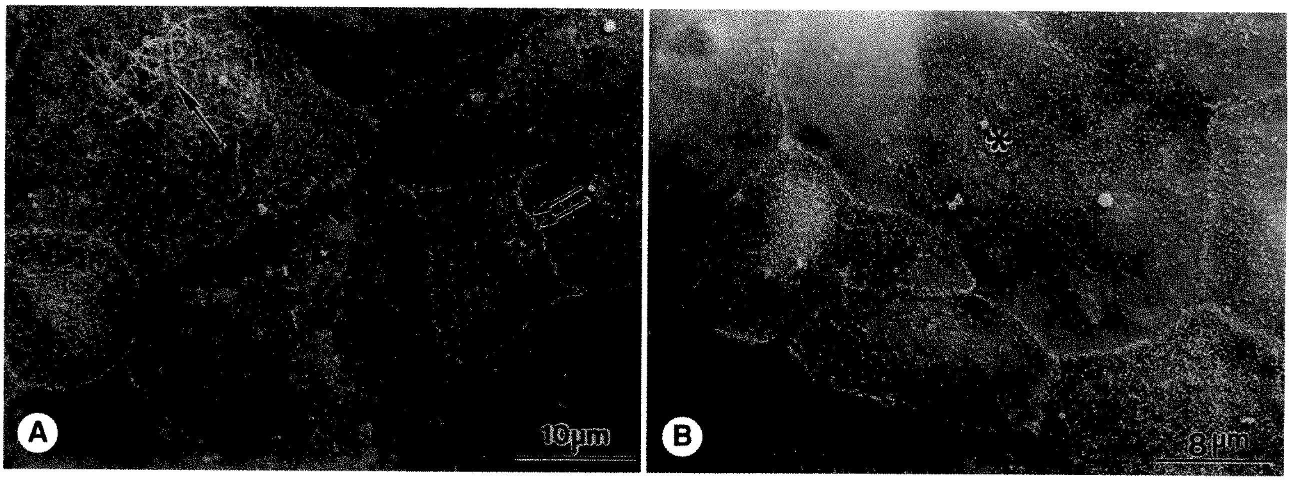

Fig 3. Scanning electron photomicrographs of CMEE-1 cells showing cell size differences. A,B) Numerous microvilli of different lengths (0.1 to 3 µm) are observed. Extremely long microvilli (single arrow) appear in clusters intermixed with short microvilli. Density of microvilli varies from cell to cell. Distinct margins between cells (double arrows) show different sizes of CMEE-1 cells and uneven distribution of microvilli on large cell (asterisk).

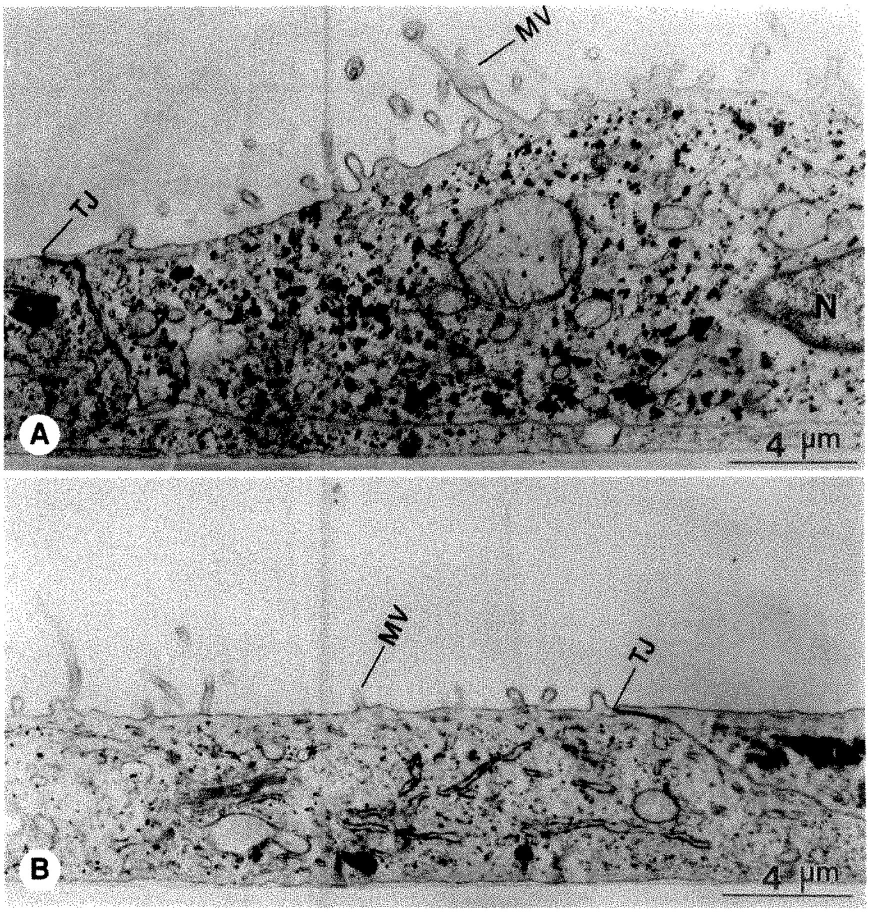

Fig 4. Transmission electron photographs of CMEE-1 cells. A) Distinct tight junction (TJ) with neighboring cells and numerous microvilli of different lengths are shown. Some of microvilli display branched shape (MV). In some areas, CMEE-1 cells appear in 2 cell layers. N — nucleus. B) CMEE-1 cells in monolayer with well-developed tight junctions (TJ) and microvilli (MV) of different lengths are shown.

positive for monospecific anti-cytokeratin antibodies 6B10, CY-90, and LDS-68. They were also positive for broad-spectrum antibodies C-11 and K8.13 and an anti-cytokeratin antibody mixture of C-11, PCK-26, CY-90, M20, and others (Table 1). These antibodies recognize cytokeratin polypeptides 1, 4-8, 10, 11, 13, 18, and 19. Neither group of cells reacted with anti-cytokeratin antibodies CK-B1, CK-E3, K4.62, PCK-26, K8.12, K8.60, and M20. These antibodies recognized cytokeratin polypeptides 1, 5, 6, 8, 10, 11, 13-17, and 19. Taken together, cytokeratins 4, 7, and 18 were positively identified in both CMEE-1 cells and control parent cells. With the vimentin antibody, the immortalized cells showed no staining, while the Vero cells showed strong positive staining (Fig 5). The immortalized cells showed positive staining in the nuclei, while the parent control cells did not show staining by antibodies against SV40 large T antigen and p53 (Fig 6).

Colony Formation in Soft Agar. After seeding of the CMEE-1 cells and the parent control cells in soft agar for 21 days, neither group of cells grew or formed colonies, but the positive control cells — human epidermal carcinoma cells (KB) — formed numerous colonies.

Western Blot. In Western blots of the cell line, a 94 kd band was observed when a monoclonal antibody against SV40 large T antigen was used, whereas a 53 kd band was seen with a monoclonal antibody against p53 (Fig 7).

Chromosomal Analysis. Karyotypic analysis of the CMEE-1 cell line was performed at passage 21 (Fig 8). The cells showed normal hyperdiploid chinchilla chromosomes with single X and Y chromosomes; 97% of the chromosome counts were in the 72 to 78 range (2N = 64). In addition, the cells showed a pair of submetacentrics with prominent satellites.[29] Cells other than CMEE-1 were not detected in the culture by cytogenetic evaluation.

DISCUSSION

Primary or serial culture of large quantities of chinchilla middle ear epithelial cells is difficult because of the short life span and limited proliferative capacity of the cells. The probability of fibroblast contamination further hampers the process when a large number of cells are cultured over long periods of time.[5,6] This is a major limitation in studying the cellular and molecular mechanisms of pathogenesis of otitis media. In order to overcome this limitation and provide a useful tool for studies of cell-cell interac-

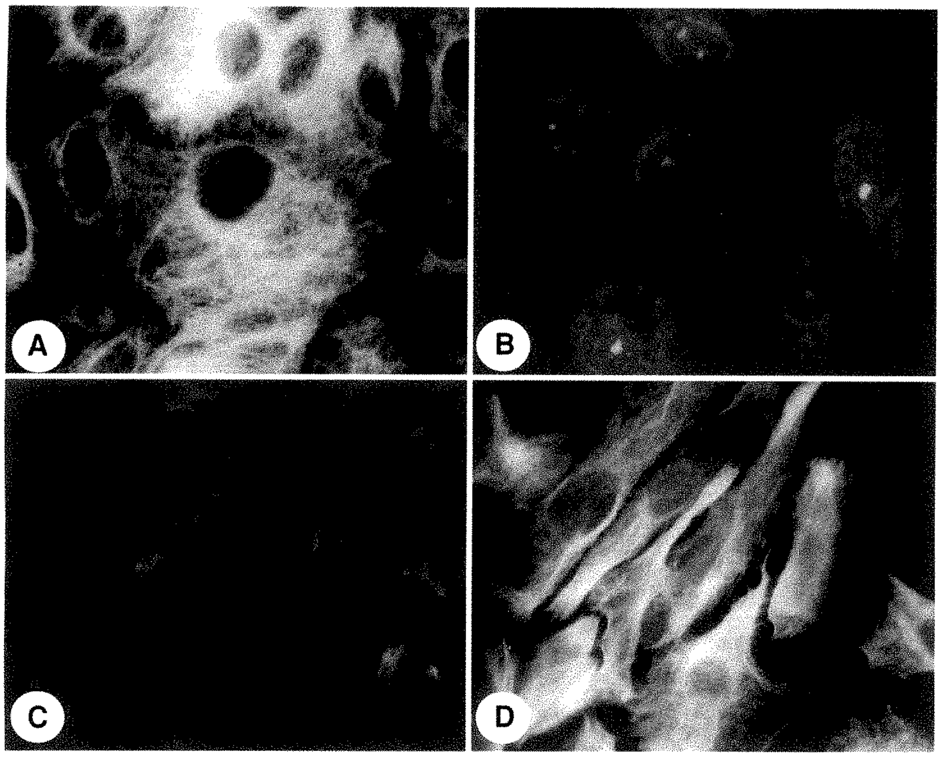

Fig 5. Indirect immunofluorescence microscopy. A) Positive labeling of CMEE-1 cells with pan-cytokeratin antibody (C-11). B) Negative labeling of CMEE-1 cells with vimentin antibody (V9). C) Negative labeling of Vero cells with pan-cytokeratin antibody (C-11). D) Positive labeling of Vero cells with vimentin antibody.

tion between the infectious bacteria and middle ear epithelial cells, we have successfully immortalized chinchilla middle ear epithelial cells by infection with Ad12-SV40 hybrid virus.

The Ad12-SV40 hybrid virus has been used to immortalize several cell types from various origins.[9-11,30-34] The SV40 large T antigen plays an important role in the immortalization. The SV40 large

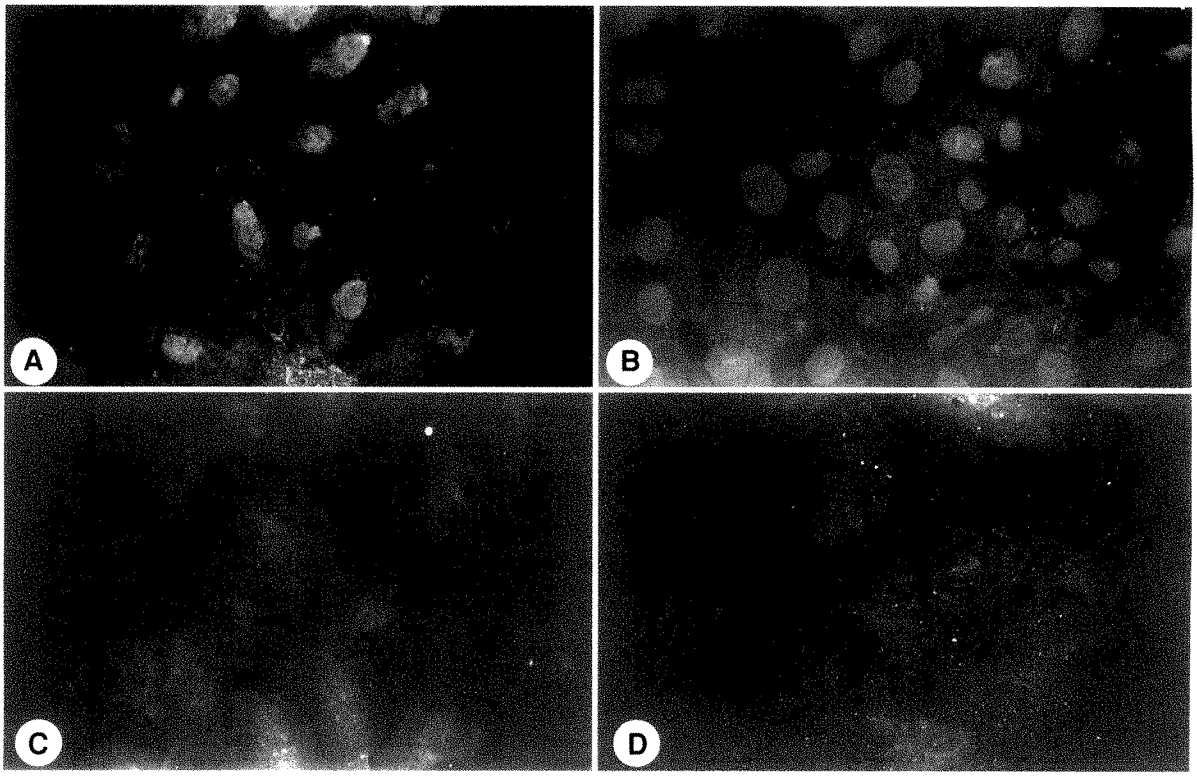

Fig 6. Immunofluorescence microscopy for expression of SV40 large T antigen and p53 (original ×630). A) Positive labeling of SV40 large T antigen and B) positive labeling of p53 in nuclei of CMEE-1 cells. C) Negative labeling of SV40 large T antigen and D) negative labeling of p53 in nuclei of non–virus-infected parent cells.

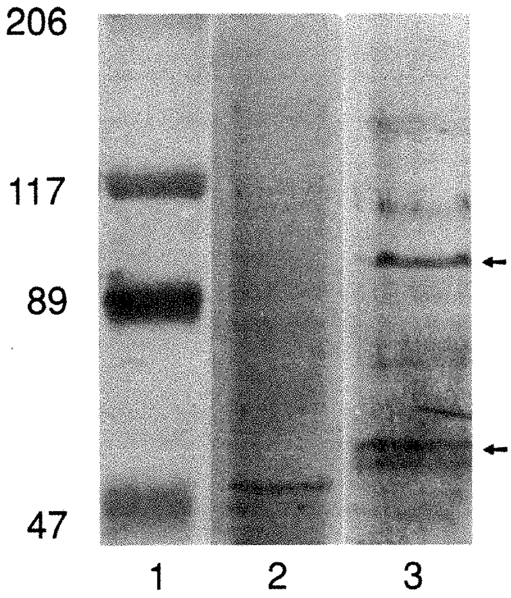

Fig 7. Western blot analysis of CMEE-1 cells; 53 kd band (arrow) was detected with monoclonal antibody against p53, and 94 kd band (arrow) was labeled with monoclonal antibody against SV40 large T antigen. Lane 1 — molecular weight marker; lane 2 — immunoblot with antibody against p53; lane 3 — immunoblot with antibody against SV40 T antigen.

T antigen binds to the protein products of the tumor suppressor gene p53, which remains up-regulated in transformed cells and appears to be essential for extending the proliferative potential of human fibroblast cells.[35] The immortalized CMEE-1 cells yielded cultures with expression of SV40 large T antigen and p53 in the nuclei. The fact that the 94 kd band labeled with SV40 antibody and the 53 kd band labeled with p53 antibody were observed in Western blots of CMEE-1 cells indicates that both SV40 large T antigen and p53 protein are expressed in these cells. These results indicate that the SV40 large T antigen and the enhanced levels of p53 expression could be responsible for the immortalizing process or maintaining the extended life span of the CMEE-1 cells.

Typically, rodent cells isolated from normal tissue will complete a given number of generations in vitro, display a decreased growth potential with increasing passages, eventually stop growing, and die. As described by Nakamura et al,[6] the growth rate of normal chinchilla middle ear epithelial cells in serial culture slowed after the 7th passage, and by the 11th passage, no further proliferation was observed. Although they maintained a continued growth at low passages, the parent control cells displayed lower growth potential from the 4th passage and eventually underwent senescence and died by the 8th passage. In contrast, the CMEE-1 cells displayed an enhanced growth rate from the 4th passage in comparison to the uninfected control cells, as shown on the growth curves. In addition, the CMEE-1 cells continued to proliferate through 42 passages over 10 months. The cells did not grow and form colonies in soft agar, nor did they form foci on the confluent monolayer of cells. Furthermore, chromosomal analysis revealed that the CMEE-1 cell line consisted of normal male hyperdiploid chinchilla cells.

Cytokeratin and vimentin are 2 of 5 intermediate filament systems that are prominent components of the cytoskeleton. Cytokeratin, which has 19 distinct cytokeratin polypeptides in human epithelial cells, is specific for epithelial cells, while vimentin is typical of mesenchymal cells[36] such as fibroblasts. Although the expression of cytokeratin in human epithelial cells has been widely reported, there are very few reports demonstrating expression of cytokeratin in chinchilla middle ear epithelial cells or other epithelium. Amesara et al[7] reported that chinchilla middle ear epithelial cells reacted with cytokeratin anti-

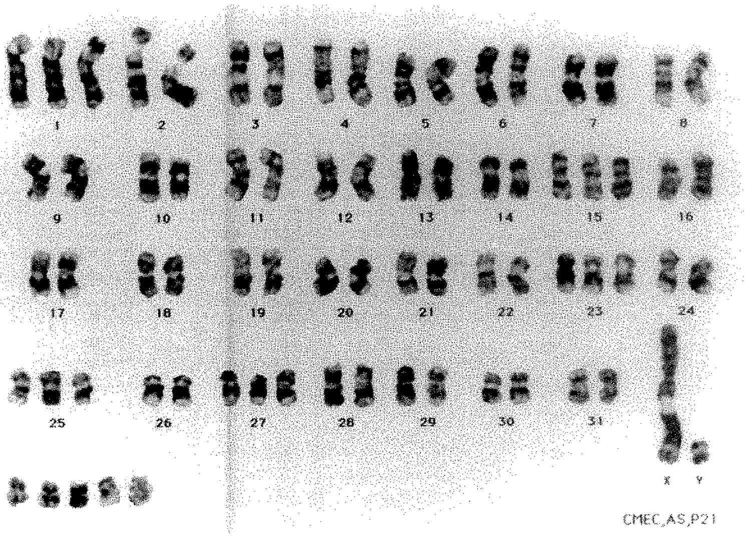

Fig 8. Chromosome photograph. CMEE-1 cells showed normal hyperdiploid chinchilla chromosomes with single X and Y chromosomes, and 97% of chromosome counts were between 72 and 78 (2N = 64).

body K8.13, which has a broad spectrum of reactivity against cytokeratin polypeptides 1, 5-8, 10, 11, and 18, and cytokeratin antibody CK-8, which is a monospecific antibody against cytokeratin polypeptide 8. Sano et al[17] reported that the broad-spectrum cytokeratin antibodies K8.13, PKK-2, and LU-5, as well as monospecific cytokeratin antibodies CK-7, CK-8, and CK-18, were positive in chinchilla middle ear simple epithelium.

In the current study, both parent control cells and CMEE-1 cells were positive for cytokeratin polypeptides 4, 7, and 18. The results were strongly supported by the staining of monospecific cytokeratin antibodies 6B10, LDS-68, and CY-90 in both cells. 6B10 recognizes cytokeratin polypeptide 4, LDS-68 recognizes cytokeratin polypeptide 7, and CY-90 recognizes cytokeratin polypeptide 18. On the other hand, neither the CMEE-1 cells nor the parent control cells showed vimentin staining, while the Vero cells showed strong staining with the vimentin antibody. These results indicate that this cell line originated from epithelial cells with no fibroblast contamination.

Cytokeratin polypeptide 8 could not be detected in either the CMEE-1 or parent control cells in this study. This result is different from that obtained by Amesara et al,[7] perhaps because of the difference in sources of monoclonal antibodies used in these 2 studies. Conflicting results of expression of cytokeratin polypeptide 8 on human middle ear epithelia with monoclonal antibodies from different clones has been reported. Van Blitterswijk et al[37] used M20, while Broekaert et al[38] used LE41 — both of which are monospecific antibodies against cytokeratin polypeptide 8. However, M20 showed a positive reaction, while LE41 showed a negative reaction, with human middle ear epithelium. Moreover, Bartek et al[39] reported different staining patterns with CK-19 in normal human epithelia with 3 monospecific monoclonal antibodies. So, the difference in expression of cytokeratin polypeptide 8 between our study and that of Amesara et al[7] could be due to differences in the antibodies used. Negative staining with cytokeratin antibody PCK-26 in the present study also differs from the results of other investigators, who reported this antibody to recognize cytokeratin polypeptides 1, 5, 6, and 8.[19] It is also possible that the negative reaction of M20 on the CMEE-1 and parent control cells may be a false-negative reaction caused by the masking of cytokeratin epitope as described by Sano et al[17] and Bartek et al.[39]

In morphological studies, the CMEE-1 cells showed a polygonal shape with tight junctions, monolayer growth, cobblestone-like appearance, numerous microvilli, rare dome formation, and absence of ciliated cells consistent with the prior description by Nakamura et al[6] of the morphology of serially cultured chinchilla middle ear epithelium. This supports our finding that the CMEE-1 cell line maintains morphological characteristics of cultured chinchilla middle ear epithelial cells.

In summary, the chinchilla middle ear epithelial cell line was successfully established by infection with the Ad12-SV40 hybrid virus. The cell line retains differentiated characteristics of normal chinchilla middle ear epithelial cells and has an extended life span and an enhanced proliferative ability without potential for malignant growth. This cell line will be useful for studying the normal biology of the chinchilla tympanal epithelial cells, as well as the cellular and molecular mechanisms involved in the pathogenesis of experimental otitis media.

ACKNOWLEDGMENTS — We thank Marianne Parrakal and Drs Mauricio Kurc and Decio Pinto for their invaluable assistance in electron microscopy. We are also indebted to Safura Babu-Khan for critically reviewing and providing invaluable assistance in the preparation of the manuscript, and to Gail Poulson for word processing.

REFERENCES

1. Giebink GS. The pathogenesis of pneumococcal otitis media in chinchillas and the efficacy of vaccination in prophylaxis. Rev Infect Dis 1981;3:342-52.

2. Green BA, Doyle WJ, Cowell JL. Chinchilla model of experimental otitis media for study of nontypeable *Haemophilus influenzae* vaccine efficacy. Methods Enzymol 1994;235:59-68.

3. Suzuki K, Bakaletz LO. Synergistic effect of adenovirus type 1 and nontypeable *Haemophilus influenzae* in a chinchilla model of experimental otitis media. Infect Immun 1994;62:1710-8.

4. van Blitterswijk CA, Ponec M, van Muijen GN, Wijsman MC, Koerten HK, Grote JJ. Culture and characterization of rat middle-ear epithelium. Acta Otolaryngol (Stockh) 1986; 101:453-66.

5. Nakamura A, DeMaria TF, Lim DJ, van Blitterswijk CA. Primary culture of chinchilla middle ear epithelium. Ann Otol Rhinol Laryngol 1991;100:774-82.

6. Nakamura A, DeMaria TF, Lim DJ, Arya G, van Blitterswijk CA. Serial culture and characterization of the chinchilla middle ear epithelium. Ann Otol Rhinol Laryngol 1991;100: 1024-31.

7. Amesara R, Kim Y, Sano S, Harata T, Juhn SK. Primary cultures of middle ear epithelial cells from chinchilla. Eur Arch Otorhinolaryngol 1992;249:164-7.

8. Harata T, Kim Y, Juhn SK, Sakakura Y. Biochemical characterization of mucous glycoproteins secreted by *in vitro* chinchilla middle ear epithelial cells. Eur Arch Otorhinolaryngol 1993;250:182-5.

9. Herman P, Cassigena R, Friedlander G, et al. Middle ear

cell line that maintains vectoral electrolyte transport. J Cell Physiol 1993;154:615-22.

10. Herman P, Yen PT, Tu TY, et al. Pathophysiology of middle ear epithelium: a new role for prostaglandin E2. Am J Otolaryngol 1994;15:258-66.

11. Ueyama S, Jin S, Rhim JS, Lim DJ. Immortalization of rat middle ear epithelial cells. In: Lim DJ, Bluestone CD, Casselbrant M, Klein JO, Ogra PL, eds. Proceedings of the Sixth International Symposium on Recent Advances in Otitis Media. Hamilton, Canada: BC Decker Inc, 1996:120-2.

12. Andley UP, Rhim JS, Chylack LT Jr, Fleming TP. Propagation and immortalization of human lens epithelial cells in culture. Invest Ophthalmol Vis Sci 1994;35:3094-102.

13. Caselitz J, Walther B, Wustrow J, Seifert G, Weber K, Osborn M. A monoclonal antibody that detects myoepithelial cells in exocrine glands, basal cells in other epithelia and basal and suprabasal cells in certain hyperplastic tissues. Virchows Arch A Pathol Anat Histopathol 1986;409:725-38.

14. Wetzels RHW, Kuijpers HJH, Lane EB, et al. Basal cell–specific and hyperproliferation-related keratins in human breast cancer. Am J Pathol 1991;138:751-63.

15. Troianovskii SM, Krutovskikh VA, Bannikov GA. Production and characteristics of monoclonal antibodies against individual prekeratins in simple types of rat epithelium [in Russian; abstract in English]. Biull Eksp Biol Med 1986;101:733-6.

16. Gigi-Leitner O, Geiger B, Levy R, Czernobilsky B. Cytokeratin expression in squamous metaplasia of the human uterine cervix. Differentiation 1986;31:191-205.

17. Sano S, Schachern PA, Haruna S, Paparella MM. Cytokeratin patterns of normal middle ear epithelia in humans, cats, and chinchillas. Ann Otol Rhinol Laryngol 1994;103:227-34.

18. Van Muijen GNP, Ruiter DJ, Warnaar SO. Coexpression of intermediate filament polypeptides in human fetal and adult tissues. Lab Invest 1987;57:359-69.

19. Lane EB, Rugg EL, Navsaria H, et al. A mutation in the conserved helix termination peptide of keratin 5 in hereditary skin blistering. Nature 1992;356:244-6.

20. Lane EB, Alexander CM. Use of keratin antibodies in tumor diagnosis. Semin Cancer Biol 1990;1:165-79.

21. Moll I, Moll R. Comparative cytokeratin analysis of sweat gland ducts and eccrine poromas. Arch Dermatol Res 1991;283:300-9.

22. Leube RE, Bader BL, Bosch FX, Zimbelmann R, Achtstatter T, Franke WW. Molecular characterization and expression of the stratification-related cytokeratins 4 and 15. J Cell Biol 1988;106:1249-61.

23. Van Muijen GNP, Ruiter DJ, Franke WW, et al. Cell type heterogeneity of cytokeratin expression in complex epithelia and carcinomas as demonstrated by monoclonal antibodies specific for cytokeratin Nos. 4 and 13. Exp Cell Res 1986;186:97-113.

24. Southgate J, Williams HK, Trejdosiewicz LK, Hodges GM. Primary culture of human oral epithelial cells. Growth requirements and expression of differentiated characteristics. Lab Invest 1987;56:211-23.

25. Lifschitz-Mercer B, Fogel M, Kushnir I, Czernobilsky B. Chorangioma. A cytoskeletal profile. Int J Gynecol Pathol 1989;8:349-56.

26. Bartek J, Vojtesek B, Staskova Z, et al. A series of 14 new monoclonal antibodies to keratin: characterization and value in diagnostic histopathology. J Pathol 1991;164:215-24.

27. Osborn M, Debus E, Weber K. Monoclonal antibodies specific for vimentin. Eur J Cell Biol 1984;34:137-43.

28. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of protein polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci U S A 1979; 76:4350-4.

29. Hsu TC, Benirschke K. An atlas of mammalian chromosomes. Vol 1. New York, NY: Springer-Verlag, 1967.

30. Arora N, Min KU, Costa JJ, Rhim JS, Metcalfe DD. Immortalization of mouse bone marrow–derived mast cells with Ad12-SV40 virus. Int Arch Allergy Immunol 1993;100:319-27.

31. Zeitlin PL, Lu L, Rhim J, et al. A cystic fibrosis bronchial epithelial cell line: immortalization by adeno-12-SV40 infection. Am J Respir Cell Mol Biol 1991;4:313-9.

32. Rhim JS, Jay G, Arnstein P, Price FM, Sanford KK, Aaronson SA. Neoplastic transformation of human epidermal keratinocytes by AD12-SV40 and Kirsten sarcoma viruses. Science 1985;227:1250-2.

33. Scholte BJ, Kansen M, Hoogeveen AT, et al. Immortalization of nasal polyp epithelial cells from cystic fibrosis patients. Exp Cell Res 1989;182:559-71.

34. Jambrosic J, Mancianti ML, Ricciardi R, Sela BA, Koprowski H, Herlyn M. Transformation of normal human melanocytes and non-malignant nevus cells by adenovirus 12-SV40 hybrid virus. Int J Cancer 1989;44:1117-23.

35. Bryan TM, Reddel RR. SV40-induced immortalization of human cells. Crit Rev Oncog 1994;5:331-57.

36. Moll R. Diversity of cytokeratin in carcinoma. Acta Histochem Suppl 1987;34:37-44.

37. van Blitterswijk CA, Grote JJ, Lutgert RW, et al. Cytokeratin patterns of tissues related to cholesteatoma pathogenesis. Ann Otol Rhinol Laryngol 1989;98:635-40.

38. Broekaert D, Cornille A, Eto H, et al. A comparative immunohistochemical study of cytokeratin and vimentin expression in middle ear mucosa and cholesteatoma, and in epidermis. Virchows Arch A Pathol Anat Histopathol 1988;413:39-51.

39. Bartek J, Bartkova J, Taylor-Papadimitriou J, et al. Differential expression of keratin 19 in normal human epithelial tissues revealed by monospecific monoclonal antibodies. Histochem J 1986;18:565-75.

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EFFECT OF CARBOGEN INHALATION ON PERIPHERAL TISSUE PERFUSION AND OXYGENATION IN PATIENTS SUFFERING FROM SUDDEN HEARING LOSS

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The effects of repeated carbogen inhalation on peripheral tissue perfusion and oxygenation were assessed in 5 patients suffering from sudden hearing loss by means of continuously measured subcutaneous tissue oxygen and carbon dioxide tension, transcutaneous oxygen tension, laser Doppler red cell flux, and fingertip temperature. The subcutaneous oxygen tension increased clearly during the carbogen inhalation periods, and also, a smaller increase in subcutaneous carbon dioxide tension was simultaneously noticed. The changes in transcutaneous oxygen tension were even greater and the latency was shorter as compared with the subcutaneous gas tensions. The laser Doppler measurements showed no signs of vasoconstriction during the study. In conclusion, carbogen inhalation increases peripheral tissue oxygenation without microvascular vasoconstriction and with only a minor retention of carbon dioxide.

KEY WORDS — carbogen, oxygenation, peripheral tissue perfusion, sudden hearing loss.

INTRODUCTION

Sudden sensorineural hearing loss (S-SNHL) is a sudden or rapidly progressive, partial or complete, typically unilateral sensorineural impairment that has no known specific etiologic factor.[1] Etiologic studies have led to 4 main theories: circulatory disturbances, viral infection, membrane rupture, and autoimmune processes. Therefore, the treatment modalities are mostly based on attempts to improve inner ear oxygenation and/or immunosuppressive therapy, to suppress either the effects of an autoimmune agent or secondary effects following a viral infection.

Carbogen inhalation therapy, in which patients breathe a gas mixture of 5% carbon dioxide (CO_2) in 95% oxygen (O_2) by mask, is used to improve inner ear oxygenation.[2] Carbogen inhalation therapy has promising results in comparison with other treatments for S-SNHL.[3]

Fisch et al[4] reported O_2 tension in human perilymph to be increased by inhalation of a carbogen gas mixture, and Kallinen et al[5] showed cochlear blood flow (CBF) in guinea pigs to be increased by carbogen inhalation, while 100% O_2 inhalation caused a decrease in CBF. In the same study, the skin blood flow was also measured, showing similar results. Miller et al[6] could not show that CBF was increased by carbogen inhalation in humans.

Clinical monitoring of peripheral tissue perfusion has been made easier and more accurate by several advanced methods now available. These include continuous recordings of subcutaneous tissue O_2 tension ($Psco_2$),[7-9] registration of skin red cell flux (RCF) by laser Doppler flowmetry,[10-14] and transcutaneous Po_2 tension ($Ptco_2$) monitoring,[15-17] as well as measurement of skin temperature.

In the present study, these methods were employed to investigate the response of peripheral tissue perfusion and oxygenation to carbogen inhalation, which is clinically used in the treatment of patients suffering from S-SNHL.

MATERIAL AND METHODS

Patients suffering from S-SNHL were hospitalized at the Department of Otorhinolaryngology, Turku University Central Hospital, for audiological and otoneurologic examinations. As reported earlier,[3] the treatment regimen consisted of carbogen inhalation therapy and betahistine hydrochloride. The carbogen inhalation therapy in the clinical setting consisted of 4 days of 6 to 8 daily inhalation periods, each of 20 to 30 minutes' duration; a mask was used to cover the nose and mouth. The betahistine hydrochloride was given 8 mg perorally 3 times a day.

The clinical material in this study consisted of 5 S-SNHL patients (3 men and 2 women) with a mean age of 45 years (range 23 to 70 years). All patients were in otherwise good health. They took no medications; 1 patient was a smoker (obviously, they did

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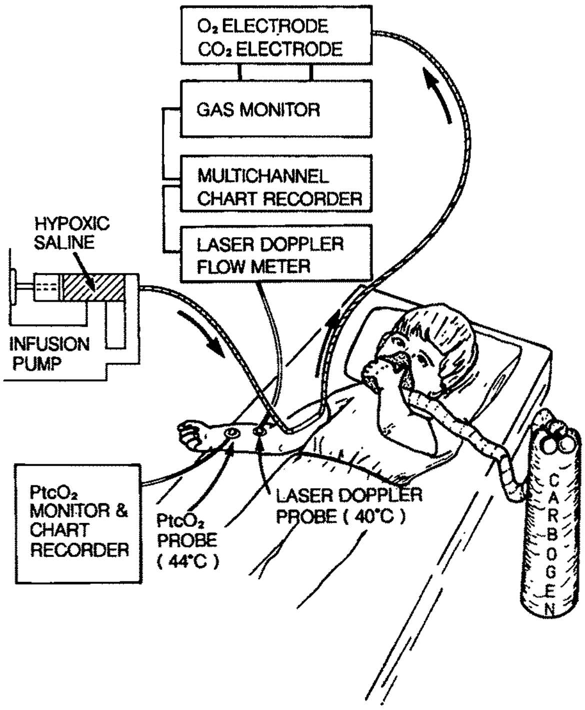

Fig 1. Measurement of subcutaneous PO2 and PCO2, transcutaneous PO2 (PtcO2), and laser Doppler skin red cell flux.

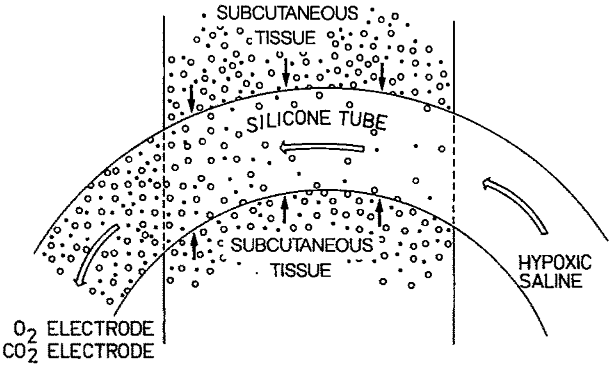

Fig 2. Equilibration of subcutaneous PO2 and PCO2 gas tensions with hypoxic saline.

not smoke during the experiments), and 1 used oral contraceptives. They did not receive betahistine during the experiments.

Peripheral circulation was monitored as follows. The peripheral skin temperature was registered from the index finger. The Ptco2, the Psco2, the subcutaneous CO2 tension (PscCO2), and the RCF were continuously recorded. The Ptco2 was registered in the right antebrachial area by means of a probe heated to 44°C with an oxygen monitor (7850A, Hewlett-Packard, Boeblingen, Germany). The Ptco2 electrode was calibrated immediately before application with a zeroing solution for the zero point and with room air for gain. A 10-minute interval for warm-up and stabilization of the electrode on the skin was allowed before reading the Ptco2 values. During the actual measurement, recalibration and reapplication were carried out only in the case of inadvertent loosening of the electrode.

The Psco2 and the PscCO2 were measured in the right upper arm by means of an implanted silicone tonometer, which was implanted under local anesthesia by means of an atraumatic needle on the second day of the carbogen inhalation therapy, and all the recordings were done 3 to 5 days later, that is, as soon as the subcutaneous space had adapted to the tube. During the measurement, the tonometer tube was perfused with hypoxic saline solution that equilibrated with the Po2 and the Pco2 of the surrounding tissue due to the high O2 and CO2 permeability of the tonometer wall. The equilibrated fluid was then directed to an O2 electrode in a blood gas analyzer connected to a chart recorder.

The RCF was measured in the right antebrachial area with a laser Doppler flowmeter (PeriFlux PF2, Perimed AB, Stockholm, Sweden) with a probe heated to 40°C.[15-17] The output was initially set to 5.0 V on a 10-V scale. This method was described earlier by

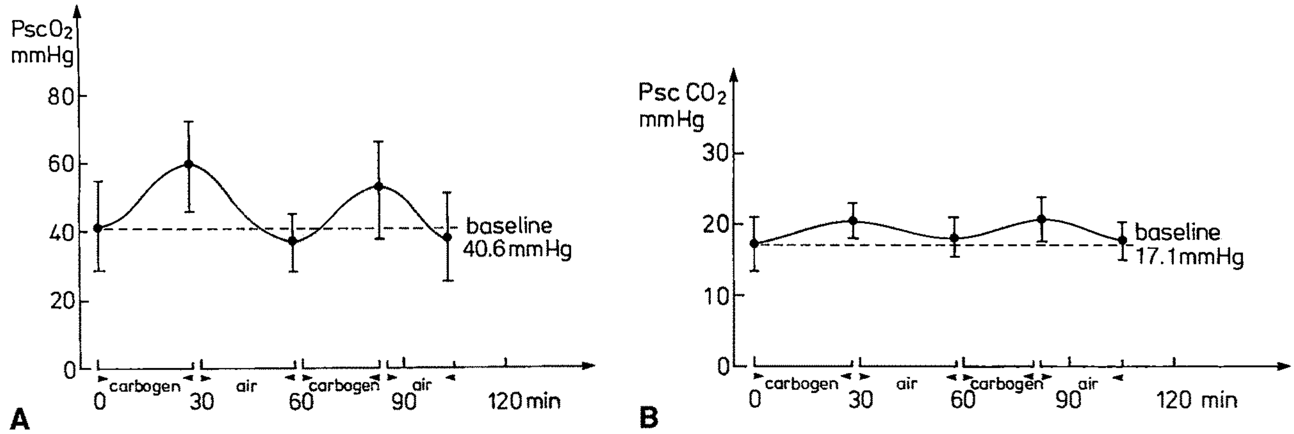

Fig 3. Subcutaneous A) PO2 and B) PCO2 during repeated carbogen and room air inhalation. Results are given as mean ± 95% confidence interval in 5 patients.

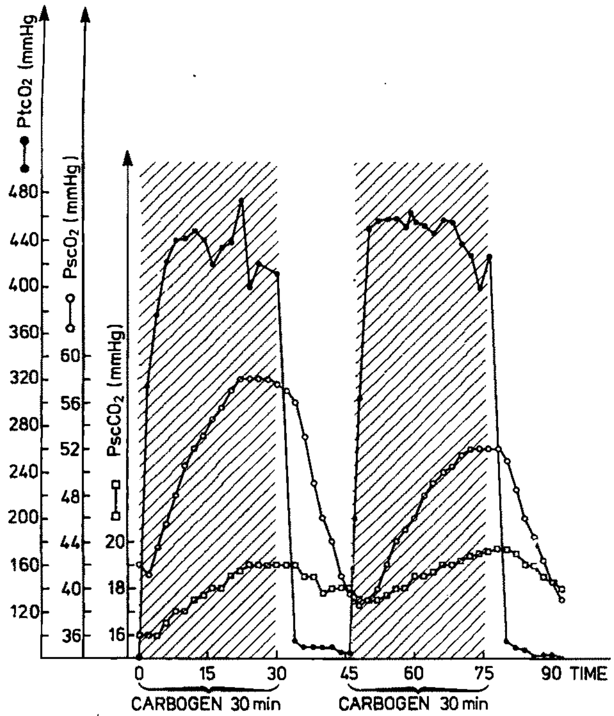

Fig 4. (Patient II) Subcutaneous PO2 (PscO2; solid circles) and PCO2 (PscCO2; squares) as well as transcutaneous PO2 (PtcO2; open circles) in 23-year-old woman during repeated carbogen and room air inhalation.

Kuttila et al.[18]

The experimental arrangement is shown in Figs 1 and 2. During the study period, the patients breathed carbogen gas mixture through a mask for 30 minutes. This was followed by 30 minutes of breathing room air and then another 30 minutes of breathing carbogen.

RESULTS

Figure 3A shows our findings of baseline PscO2 and changes in PscO2 observed with carbogen inhalation. The mean baseline on PscO2 of the 5 patients studied was 40.6 ± 4.7 mm Hg, and at the first carbogen inhalation period, the PscO2 increased to 58.8 ± 6.6 mm Hg (Fig 3A). Then, during room air breathing, the PscO2 decreased to 36.7 ± 3.1 mm Hg, and it increased to 50.6 ± 5.5 mm Hg at the second carbogen breathing period. Following the second carbogen inhalation the patients' PscO2 decreased to 37.6 ± 4.2 mm Hg.

SUBJECT'S SEX, AGE, AND HEARING LEVEL BEFORE AND AFTER TREATMENT

			Hearing Level (dB)*	
Patient	Sex	Age (y)	At Diagnosis	At Control
I	M	53	83	28
II	F	23	100	90
III	M	70	81	15
IV	M	35	87	48
V	F	43	25	8

*Average of 500, 1,000, and 2,000 Hz.

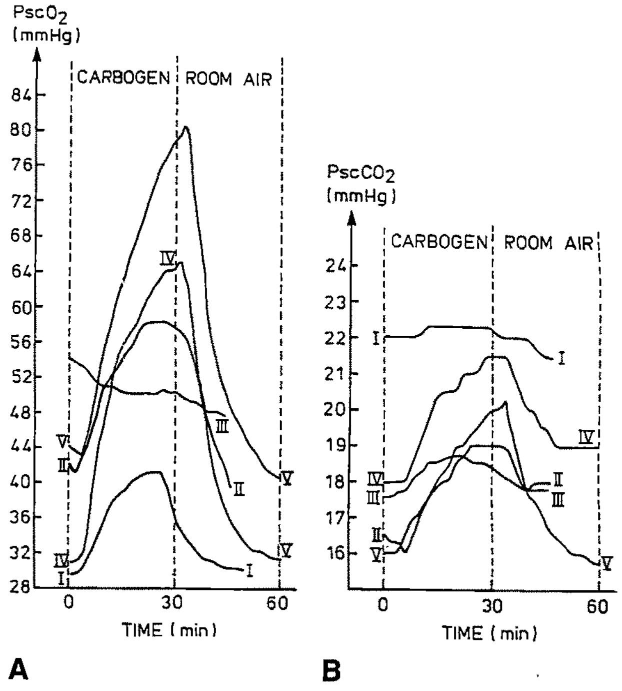

Fig 5. Individual subcutaneous A) PO2 and B) PCO2 changes during carbogen inhalation. Roman numerals are patient numbers.

Correspondingly, the PscCO2 tension (Fig 3B) increased from the mean baseline of 17.1 ± 1.2 mm Hg to 20.3 ± 0.7 mm Hg at the first carbogen inhalation period. During the room air breathing break, it recovered to 18.2 ± 3.9 mm Hg; it increased in the second carbogen period to 21.0 ± 1.2 mm Hg, and recovered again afterward to 18.1 ± 1.0 mm Hg.

The changes of PscO2 and PscCO2 in patient II, a 23-year-old woman, are shown in Fig 4, as well as the simultaneous PtcO2 changes. The change in the PtcO2 is bigger and the latency is shorter compared with those of the PscO2.

The individual changes in hearing levels that occurred after the treatment with carbogen are shown in the Table. Figure 5 demonstrates the individual changes in PscO2 and PscCO2 during the first carbogen inhalation period.

The laser Doppler readings and fingertip temperatures remained stable throughout the study period, indicating that no vasoconstriction was induced.

DISCUSSION

Carbogen inhalation therapy has promising results in the treatment of patients suffering from S-SNHL.[2,3] Breathing the gas mixture with the mask is a convenient method of administration. The patients are able, after instruction, to carry out the treatment with little or no assistance from the hospital personnel. It is a relatively safe treatment method with no contraindications, and only a few patients feel a slight discomfort with using the mask.

In the present study, breathing of the carbogen mixture constantly increased the peripheral PO_2 measured both subcutaneously and transcutaneously. The latency of the observed change in the PO_2 is short, both at the beginning of the carbogen inhalation and after finishing it. Repeated breathing periods did not affect the absorption of the gases, and the effect during the second breathing period was similar to the response to the first one.

No changes were observed in the RCF measured with laser Doppler flowmetry during the gas administration, and this finding indicates that no vasoconstriction occurred in the blood vessels, whereas we know from previous observations that pure O_2 breathing would cause such a change. This vasoconstriction is presumably avoided by the 5% CO_2 in the gas mixture. This CO_2 concentration causes only a small increase in $PscCO_2$ that is harmless to the patients.

The described method for measuring the changes of the gas concentrations in peripheral tissues is reliable and easy to perform.[18] It has also been shown that the observed changes in the subcutaneous space correlate with the changes measured from the inner ear in experimental animals[5] and in humans.[6]

Our preliminary results in the treatment of S-SNHL patients with this method show promising results. Further studies in larger series are required to verify these results in patients whose S-SNHL is due to vascular and other damage in the inner ear. Another application of this therapy could be in overcoming the problem of hypoxia in solid malignant tumors during radiotherapy.

REFERENCES

1. Byl F. Sudden hearing loss: eight years' experience and suggested prognostic table. Laryngoscope 1984;94:647-61.

2. Fisch U. Management of sudden deafness. Otolaryngol Head Neck Surg 1983;91:3-8.

3. Kallinen J, Laurikainen E, Laippala P, Grénman R. Sudden deafness: a comparison of anticoagulant therapy and carbogen inhalation therapy. Ann Otol Rhinol Laryngol 1997;106: 22-6.

4. Fisch U, Murata K, Hossli G. Measurement of oxygen tension in human perilymph. Acta Otolaryngol (Stockh) 1976; 81:278-82.

5. Kallinen J, Didier A, Miller J, Nuttall A, Grénman R. The effect of CO2- and O2-gas mixtures on laser Doppler measured cochlear and skin blood flow in guinea pigs. Hear Res 1991;55:255-62.

6. Miller JF, Bredberg G, Grenman R, Suonpää J, Lindström B, Didier A. Measurement of human cochlear blood flow. Ann Otol Rhinol Laryngol 1991;100:44-53.

7. Niinikoski J, Hunt TK. Measurement of wound oxygen with implanted Silastic tube. Surgery 1972;71:22-6.

8. Niinikoski J, Laaksonen V, Meretoja O, Jalonen J, Inberg MV. Oxygen transport to tissue under moderate and extreme haemodilution during coronary bypass operation. Ann Thorac Surg 1981;31:134-43.

9. Heppenstall RB, Littooy FN, Fuchs R, Sheldon GF, Hunt TK. Gas tensions in healing tissues of traumatized patients. Surgery 1974;75:874-80.

10. Waxman K, Sadler R, Eisner ME, Applebaum R, Tremper KK, Mason GR. Transcutaneous oxygen monitoring of emergency department patients. Am J Surg 1983;146:35-8.

11. Huch A, Huch R, Arner B, Rooth G. Continuous transcutaneous oxygen tension measured with a heated electrode. Scand J Clin Lab Invest 1973;31:269-75.

12. Tremper KK, Shoemaker WC. Transcutaneous oxygen monitoring of critically ill adults, with and without low flow shock. Crit Care Med 1981;9:706-9.

13. Nolan LS, Shoemaker WC. Transcutaneous O2 and CO2 monitoring of high risk surgical patients during the perioperative period. Crit Care Med 1982;10:762-4.

14. Goethgen I, Jakobsen E. Transcutaneous oxygen tension measurements I. Age variation and reproducibility. Acta Anaesthesiol Scand Suppl 1978(suppl 67):66-70.

15. Stern MD. In vivo evaluation of microcirculation by coherent light scattering. Nature 1975;254:56-8.

16. Engelhart M, Kristensen JK. Evaluation of cutaneous blood flow responses by 133 xenon washout and a laser Doppler flowmeter. J Invest Dermatol 1983;80:12-5.

17. Tenland T, Salerud EG, Nilsson GE, Öberg PÅ. Spatial and temporal variations in human skin blood flow. Int J Microcirc Clin Exp 1983;2:81-90.

18. Kuttila K, Niinikoski J. Peripheral perfusion after cardiac surgery. Crit Care Med 1989;17:217-20.

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DISCOVERING DIAGNOSTIC RULES FROM A NEUROTOLOGIC DATABASE WITH GENETIC ALGORITHMS

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Data on patients with Meniere's disease, vestibular schwannoma, traumatic vertigo, sudden deafness, benign paroxysmal positional vertigo, or vestibular neuritis were retrieved from the database of otoneurologic expert system ONE for the development and testing of a genetic algorithm (GA). The accuracy of the diagnostic rules in solving the test cases was 81%, 91%, 92%, 95%, 96%, and 98% for the respective diseases. The best rules retrieved from the GA were described by a set of questions with the most likely answers. The most important questions concerned the duration of hearing loss and the occurrence of head injury. The validity and structure of the rules created with a GA can be analyzed in detail. For rare diseases, some other reasoning process can be used, for example, case-based reasoning.

KEY WORDS — artificial intelligence, diagnostic workup, dizziness, genetic algorithms.

INTRODUCTION

Genetic algorithms[1,2] are search algorithms that have their origin in natural systems. Thus, the power of genetic algorithms derives from the combined principles of Darwinian evolution and natural genetics that allow a genetic algorithm to adapt to different environments. Genetic algorithms have many advantages over traditional search algorithms, including conceptual simplicity, robustness, broad applicability, extensibility, and the potential to hybridize with other methods.[2-4]

A simple genetic algorithm[2] modifies a population consisting of solutions with selection, crossover, and mutation operators to produce a possibly better new generation, until the algorithm fulfills a terminating condition (Fig 1). In every generation, the fitness of each solution is obtained from the fitness function, which rewards the solution according to user-defined criteria. A selection operator acts as the natural selector by copying the solutions with a better fitness of higher probability into the next generation. As in nature, the solutions incapable of adapting to the environment have fewer offspring and are doomed to extinction.

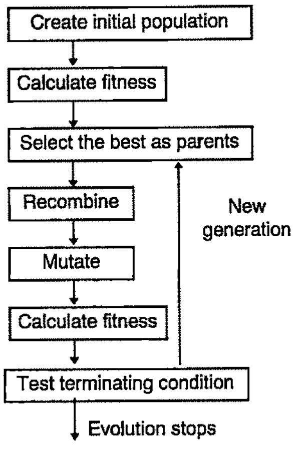

Fig 1. Simple genetic algorithm.

Selection in itself does not help the genetic algorithm to explore new areas in the search space. For this reason, new genetic material is created from the earlier solutions by means of crossover, which exchanges parts of randomly selected parents to produce better solutions. Occasionally, mutation generates random changes in the new solutions to prevent the loss of possibly useful gene combinations. Both crossover and mutation operators are applied with a user-defined probability.

The natural elegance and power of genetic algorithms have led to applications to many problem domains, including optimization of functions, scheduling, data analysis, prediction, and machine learning.[2,3] In medicine, genetic algorithms have been utilized, for example, to model the immune system[5] and to identify persons at risk of coronary artery disease.[6] In this paper, we have used the genetics-based ma-

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TABLE 1. NUMBER OF DIAGNOSES (N = 564) IN LEARNING (N = 389) AND TESTING (N = 175) SETS

Diagnosis	*Learning Set*	*Testing Set*	*Total*
Vestibular schwannoma	91	37	128
Benign paroxysmal positional vertigo	40	19	59
Meniere's disease	171	72	243
Sudden deafness	12	9	21
Traumatic vertigo	35	18	53
Vestibular neuritis	40	20	60

chine learning system Galactica[7] to discover the diagnostic rules for the 6 most frequent otologic diseases involving vertigo in a well-researched database.[8]

MATERIALS AND METHODS

Patients. The information on the patients in the database was collected from the vestibular unit of the Helsinki University Hospital. The patients referred to the vestibular laboratory filled out a questionnaire concerning their symptoms, earlier diseases, accidents, and use of tobacco and alcohol.[9] The information was stored in the otoneurologic expert system ONE.[9] From the database of ONE, we selected the 6 largest patient groups with vertigo. The diseases were Meniere's disease, vestibular schwannoma, vestibular neuritis, benign paroxysmal positional vertigo, traumatic vertigo, and sudden deafness. The diagnoses were confirmed by an experienced specialist in the field of otoneurology. We applied genetic algorithms to these solved cases to create new decision-making rules automatically from patient data. Part of the patient data were used in the learning set, and the rest in the testing set, in which the accuracy of the rule was tested (Table 1). Both sets contained patients from all the disease groups.

Parameters. The database of ONE was collected with the aid of 170 questions.[8] The information in the database can be divided into the following classes: 1) symptoms, (eg, vertigo, hearing loss, tinnitus, unsteadiness, headache, anxiety, and neurologic symptoms); 2) medical history, including the use of ototoxic drugs, head or ear trauma, noise injury, ear infections and operations, specific infections (borreliosis, chlamydia, lues), and other diseases; and 3) findings from such examinations as clinical and neurotologic examinations (saccadic and pursuit eye movements, posturography, electronystagmography, brain stem audiometry, and electrocochleography), audiometry, imaging (computed tomography, magnetic resonance imaging), and fistula tests.[8] Case history data have proven to be the most valuable in classifying the 6 otologic diseases involving vertigo.[9] Using this information, we selected 38 parameters that were valuable in the classification. Some good parameters had to omitted due to the large amount of missing information; for example, only a minority of patients underwent computed tomography or magnetic resonance imaging. The 38 parameters are presented in Table 2.

TABLE 2. PARAMETERS USED IN LEARNING PROCESS

- Age at first symptoms
- Vertigo
  - Duration of symptoms
  - Duration of vertigo attacks
  - Frequency of vertigo attacks
  - Intensity of vertigo attacks
  - Score for rotational vertigo
  - Score for floating sensation
  - Score for position-induced vertigo
  - Unsteadiness
  - Sudden falls
- Hearing loss
  - Duration of hearing loss
  - Type of hearing loss
  - Location of hearing loss
  - Fluctuation in hearing
- Tinnitus
  - Intensity of tinnitus
  - Duration of tinnitus
- Severity of nausea
- Light-headedness and other functional symptoms
- Ear illness
- Ear operation
- Head injury
- Ear trauma
- Noise injury
- Spontaneous nystagmus
- Electronystagmography
  - Spontaneous nystagmus
  - Caloric asymmetry
  - Response with 44°C right
  - Response with 44°C left
- Posturography
  - Eyes open
  - Eyes closed
- Pursuit eye movements
  - Gain amplitude
  - Gain latency
- Tone burst audiometry
  - 500 Hz right
  - 500 Hz left
  - 2,000 Hz right
  - 2,000 Hz left

Galactica. Galactica[7] is an inductive learning system[10] that uses a genetic algorithm to learn concepts from databases that contain examples labeled by a human supervisor as positive or negative with respect to the concept. Concepts are characterized by attributes; for example, a Fruit concept may be described by Color, Shape, and Size attributes. Concepts are presented formally with IF-THEN style rules, which consist of condition and decision parts. The condition part of the rule specifies the attribute values that an example must have in order to be classified as belonging to the concept that is shown in the decision part of the rule. For example, a rule for identifying an Apple concept might be: IF (Color = Red OR Yellow) AND (Shape = Round) AND (Size = Small) THEN Apple. Thus, other fruits, for instance, bananas or oranges, could be detected with conditions that have been modified to express their characteristics. The conditions can be described as a filter that lets the examples with the right values for attributes pass through it and traps the rest; green apples will not be permitted through even if they are round or small.

The genetic algorithm maintains a population of

TABLE 3. ACCURACY OF RULES FOR SIX OTOLOGIC DISEASES INVOLVING VERTIGO

	Testing Set					*All Cases*				
	Accuracy	*Positive Cover*		*Negative Cover*		*Accuracy*	*Positive Cover*		*Negative Cover*	
Diagnosis	*%*	*No.*	*%*	*No.*	*%*	*%*	*No.*	*%*	*No.*	*%*
VS	91	23/37	62	2/132	2	92	82/128	64	2/436	1
BPPV	96	14/19	74	2/150	1	97	45/59	76	6/505	1
MEN	81	55/72	76	16/97	16	83	186/243	77	41/321	13
SUD	95	1/9	11	0/160	0	97	4/21	19	0/543	0
TRV	92	5/18	28	0/150	0	93	14/53	26	0/511	0
VN	98	18/20	90	1/149	1	98	51/60	85	4/504	1

VS — vestibular schwannoma, BPV — benign paroxysmal positional vertigo, MEN — Meniere's disease, SUD — sudden deafness, TRV — traumatic vertigo, VN — vestibular neuritis.

candidate rules from which it selects the best to produce the new generation. The fitness of a rule is calculated from the negative (consistency) and positive (completeness) covers of the rule in a learning set and by the complexity of the rule. The highest fitness is given to a rule that covers all the positive and none of the negative examples with a minimum number of conditions.

A small number of cases (traditionally 30%) is excluded from the learning set and placed in a testing set to make the evaluation of the results objective. Accuracy reveals how well the unseen patients with the disease in question are selected by the rule and how many false positives (patients without the disease) are classified into the disease group. In other words, accuracy is the ratio of the correctly classified examples to all the examples in the testing set: Accuracy = $(e_{pos} + E_{neg} - e_{neg})/E$, where e_{pos} and e_{neg} are the positive and negative covers of the rule set, E_{neg} is the number of negative examples in the testing set, and E is the total number of examples in the testing set.

RESULTS

Galactica generated differential diagnostic rules for Meniere's disease, vestibular schwannoma, vestibular neuritis, benign paroxysmal positional vertigo, traumatic vertigo, and sudden deafness. The accuracies of the rules were calculated both from the testing set and from all the available cases (Table 3). The accuracies were close to one another. This result indicates that the testing sets were representative of the database. The slightly higher accuracies obtained from all the cases also indicate that as usual, the rule classified the learning sets better than the testing sets. However, in this study, we were mainly interested in the classification accuracy of unseen cases (prediction accuracy).

The questions from which the conditions for the rules were generated with the genetic algorithm are shown in Table 4. A condition is a question with different answer possibilities that are useful as exclusion or inclusion criteria for the disease. Each question has several answer possibilities that can form specific conditions to characterize each disease. It appeared that disease groups with only a few cases required more conditions to the rule. As a result of the learning process, the conditions in the rules were those that expressed and classified our material the best.

Population size was set experimentally at 200 members, and the number of generations was 150. The probabilities of crossover and mutation were .95 and .01, respectively.[11,12] Two of Michalski's learning operators,[13] which were both applied with a probability of .01, were used to quicken the learning process. The best member of each population was inserted into the next population without modifications to prevent the loss of valuable information. Better initial populations were generated by selecting 20% of the population randomly from the learning set.[7] The learning cycle was repeated 6 times, and the best outcome was selected to be reported.

Vestibular Schwannoma. Vestibular schwannoma could be classified correctly in 91% of the cases in the testing set after the learning process. This was a high-accuracy percentage, since the data from computed tomography or magnetic resonance imaging were not used in the classification. The rule that classified vestibular schwannoma had 9 questions concerning the duration of vertigo and hearing loss; the occurrence of head injury; the intensity of the vertigo attack; the score for positional, rotational, and floating vertigo; the intensity of nausea; and the presence of sudden falls (Table 4). Vertigo was absent in 65 patients (51%). The intensity of the vertigo attacks was commonly from mild (35%) to moderate (35%). The vertigo was rotational in 40% of the patients and floating in 43%. Rotational vertigo was experienced as stronger than the floating form. The vertiginous patients with vestibular schwannoma had significantly less nausea than patients in the other disease

TABLE 4. FREQUENCIES OF QUESTIONS THAT FORMED CONDITIONS FOR RULES FOR SIX OTOLOGIC DISEASES INVOLVING VERTIGO

Condition	*VS*	*BPPV*	*MEN*	*SUD*	*TRV*	*VN*	*Total*
Duration of hearing loss	1	1	0	1	1	1	5
Injury	1	1	1	0	1	0	4
Nausea	1	1	0	1	1	0	4
Audiometry at 500 Hz right	0	0	1	1	1	1	4
Duration of vertigo	1	0	1	1	0	0	3
Attack intensity	1	0	0	1	1	0	3
Vertigo rotational	1	0	0	1	1	0	3
Attack duration (vertigo)	0	1	0	1	1	0	3
Audiometry at 2,000 Hz right	0	1	0	0	1	1	3
Attack frequency (vertigo)	0	0	0	1	1	1	3
Hearing fluctuation	0	1	0	1	0	1	3
Sudden falls	1	0	0	1	0	0	2
Positional vertigo	1	0	0	1	0	0	2
Age at onset	0	1	0	1	0	0	2
Hearing better in left ear	0	1	0	0	0	1	2
Head trauma	0	1	0	0	1	0	2
Audiometry at 500 Hz left	0	1	0	0	0	1	2
Caloric response with 44°C right	0	0	1	1	0	0	2
Duration of tinnitus	0	0	0	1	1	0	2
Posturography eyes open	0	0	0	1	1	0	2
Gain latency (pursuit eye movements)	0	0	0	1	1	0	2
Floating vertigo	1	0	0	0	0	0	1
Hearing better in right ear	0	1	0	0	0	0	1
Posturography eyes closed	0	1	0	0	0	0	1
Intensity of tinnitus	0	0	1	0	0	0	1
Ear trauma	0	0	1	0	0	0	1
Hearing loss type	0	0	0	1	0	0	1
Caloric asymmetry	0	0	0	1	0	0	1
Unsteadiness	0	0	0	0	1	0	1
Audiometry at 2,000 Hz left	0	0	0	0	0	1	1
Total	9	12	6	18	14	8	67

VS — vestibular schwannoma, BPPV — benign paroxysmal positional vertigo, MEN — Meniere's disease, SUD — sudden deafness, TRV — traumatic vertigo, VN — vestibular neuritis. Boldface type points out most important questions. 1 — Condition is present in rule (question or some answer possibility is meaningful as exclusion or inclusion criterion), 0 — condition does not belong to rule.

groups with vertigo (Fig 2A).

Benign Paroxysmal Positional Vertigo. Benign paroxysmal positional vertigo was classified correctly in 96% of the cases. The important questions concerned the duration of the vertigo attack and details that confirmed normal hearing (audiometry findings, no fluctuation in hearing, and exclude asymmetry between the ears). The negative cover in benign positional vertigo was good: none of the patients in the other disease groups were classified as belonging to the benign paroxysmal positional vertigo group.

Meniere's Disease. The accuracy in identifying Meniere's disease was the lowest, being only 81%. The number of questions constructing the classification rule was only 6 (Table 4). The most important question was the intensity of tinnitus, which solely classified 63% of the test cases correctly. The other 5 questions in the rule were the duration of vertigo, the occurrence of head or ear injury, the response with 44°C in electronystagmography, and the audiometry finding at 500 Hz. We explored the causes for the low accuracy for Meniere's disease by separately exploiting all the subgroups (ie, all answer possibilities to each of the 6 questions) in the rule. It revealed that there were 3 subgroups that led to a better rule, with 84% accuracy. The genetic algorithm was not able to find this ultimate rule from the learning set. In the test set, 16% of the negative cases (eneg) were classified as Meniere's disease.

Sudden Deafness. Sudden deafness required the most parameters (18) to generate a rule with an accuracy of 95% in the testing set (Table 4). The small number of patients in the sudden deafness group may have been the cause of the large number of parameters in this rule. If the case number had been larger, the genetic algorithm would probably have been able to consider a rule with fewer questions. The distribution of symptoms was the widest for sudden deafness; in other words, many answer possibilities were covered for each question. The frequency and dura-

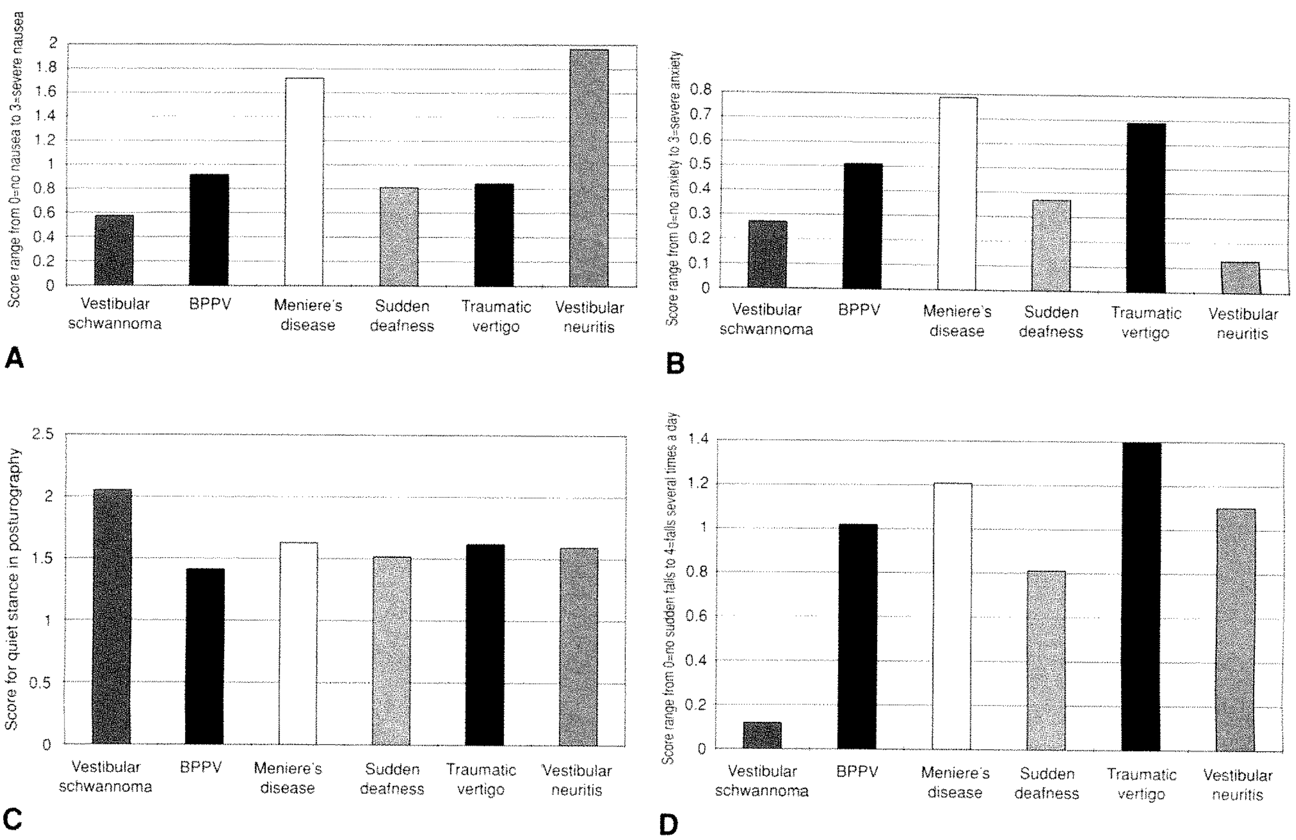

Fig 2. Mean scores for A) nausea, B) anxiety, C) quiet stance with eyes open in posturography, and D) occurrence of sudden falls. BPPV — benign paroxysmal positional vertigo.

tion of a vertigo attack had no typical characteristics. Vertigo was experienced by 86% (n = 18) of the patients. Any nausea accompanying vertigo was mild.

Traumatic Vertigo. For traumatic vertigo, 14 questions generated a rule with an accuracy of 92%. A positive response to the occurrence of head injury was the most important separate variable in the rule. Vertigo attacks appeared several times a week, lasting from 15 seconds to 5 minutes. The rule allowed more variance, however, for the description of vertigo and the accompanying symptoms than for the other diseases. The onset of hearing loss, tinnitus, and vertigo had to be related timewise. The score for anxiety was high for the patients with traumatic vertigo, but this deviating finding did not influence the composed rule (Fig 2B).

Vestibular Neuritis. The best accuracy, 98%, was reached for vestibular neuritis. Only 1 of the negative test cases was classified as vestibular neuritis. The frequency of vertigo attacks (only once) and details confirming normal hearing (normal audiometric findings, no fluctuation in hearing, and exclude asymmetry between the ears) were important in this rule.

DISCUSSION

Genetic algorithms were used to generate diagnostic rules from neurotologic data. The accuracy of the rules was above 90% for all the diseases except Meniere's disease. Meniere's disease is often difficult for the otologist to diagnose, as well. The diagnostic rules created with genetic algorithms contained the most important diagnostic questions described earlier. These 5 key questions discriminating between the diseases concern the duration of vertigo and hearing loss, the duration and frequency of vertigo attacks, and the occurrence of head trauma with an onset of symptoms.[9]

In addition, this study demonstrated that Galactica was able to perform well with a different problem with a large search space. In our earlier experiments with data on female urinary incontinence, we were able to extract accurate diagnostic rules from examples that were described with 13 binary attributes.[7] The vertigo data used in the experiments reported here were characterized by 38 attributes having from 2 to 8 possible values.

The cause of many diseases involving vertigo is unknown. The diagnosis must be based on a certain combination of clinical symptoms and signs after other possible causes have been excluded. Vestibular schwannoma is the only disease that has a specific diagnostic test, namely, magnetic resonance imaging, that confirms the diagnosis.[9] In many diseases the cause is unknown; for example, in sudden deafness several etiologic factors, such as infection or vascular causes, have been suggested.[14] Etiologic factors are therefore of little use in the diagnostic workup. The prevalence of diseases cannot be used, because common diseases are so common that all rare diseases would be lost in the reasoning. Important symptoms other than vertigo are hearing loss and tinnitus. The intensity and duration of vertigo attacks vary from one disease to another. For example, the vertigo attacks in benign paroxysmal positional vertigo are short and come with a certain head position, while in vestibular neuritis the vertigo attack can last several days.

For some diseases follow-up is necessary before the diagnosis can be confirmed. Meniere's disease can start with any of 3 major symptoms (vertigo, hearing loss, tinnitus),[9] but unless the full triad is present the diagnosis of Meniere's disease cannot be made.[15] Fluctuation in hearing level may not be typical for Meniere's disease. When the cause is known, the symptom triad is called Meniere's syndrome.[14] The cause can be, for example, an infection (lues), trauma, or autoimmune disease.[14] The words by which the patients describe their disease can also vary from patient to patient. To standardize the descriptions, we used a questionnaire for the patients in this study.[9]

Patients can have several diseases at the same time, for example, noise-induced hearing loss and vertigo. Deviant findings have to be evaluated with respect to the entire situation and all diseases. Vascular disturbances causing vertigo identical to that seen in peripheral vestibular diseases are also diagnostically difficult.[16] Patients with vestibular schwannoma and Meniere's disease have had the disease longer than patients have sudden deafness, vestibular neuritis, or benign paroxysmal positional vertigo. This difference may affect the distinction of the diseases.

The high accuracy in detecting vestibular schwannoma without the results of imaging studies is worth noting. It is partly due to the deviant findings in electronystagmography and posturography (Fig 2C). In the clinical picture, the low incidence of vertigo (49%) and the absent or mild nausea accompanying it differentiated vestibular schwannoma from the other diseases. The vestibular schwannoma patients had also had notably fewer sudden falls than the other patients (Fig 2D).

The small number of patients in rare disease groups makes the use of machine learning techniques difficult, and sometimes impossible. That is why we have to use only the 6 largest patient groups from our database.[8] In real life there are more diagnostic choices for vertigo than these 6 diseases. We are planning to test Galactica to see how well it will perform in learning rules from small diagnostic groups. An alternative solution for diagnosing diseases with only a few patients can be case-based reasoning. Pure patient cases without confounding diseases would be required for case-based reasoning.

In comparison to our earlier experiments with neural networks, the genetic algorithm proved to be better in practice, due to its capability to work with incomplete information and the symbolic structure of the learned knowledge.[17] Neural networks may work unpredictably if complete input information is not available. Moreover, the knowledge neural networks produce is nonsymbolic and difficult to understand.[18] Genetic algorithms are a quite straightforward method in comparison to the neurotologic expert system ONE. The pattern recognition method of ONE is elegant, because it is able to detect patients belonging even to rare disease groups.[19] In the pattern recognition method, the user also has the possibility to influence the decision-making process of the system.

The updating of information is easily achieved both in our neurotologic expert system ONE[19] and with genetic algorithms. A genetic algorithm can go through a large database of cases and produce new diagnostic rules in a few hours. In the pattern recognition–based expert system, the updating is done by the user when new diagnostically valuable information is available.

The behavior of the genetic algorithms depends on many control parameters, population size being one of the most important. With our problem, we found that a fairly large population produced the best results. The relatively high crossover (.95) and mutation probabilities (.01) suggested by Grefenstette[11] and Schaffer et al[12] turned out to be the best values for this problem environment. In our earlier experiments, smaller population sizes and lower probabilities for operators produced good results.[7] The difference demonstrates an unfortunate drawback for genetic algorithms, namely, the problem of optimal control parameter values.[3] Even if experimental and theoretical research has given some guidelines for selecting these values, a researcher must often make several tests to find a good parameter combination for the problem to be solved.

The development of a genetic algorithm–based learning method is at an initial stage. The produced rules are simple, being all or none, meaning that the diagnosis either fits or does not. All the questions that have been used to create a rule do not need to be present, but within the rule, all different conditions have to match. For example, a good rule for vestibular neuritis could be created with only 8 questions (Table 4). At present, all conditions are of equal value in the rules. By giving more weight to the most important questions, the accuracy of the rules might be increased.

CONCLUSION

Genetic algorithms were able to create accurate diagnostic rules for 6 diseases involving vertigo. The diagnostic rules produced with the genetic algorithms are symbolic, meaning that the rules can be comprehended. This ability makes it possible to explore the rule and verify its validity. Nevertheless, it is sometimes difficult for an expert to examine rules because of the great number of conditions. Therefore, some type of rule-pruning technique that reduces the number of conditions might make the rules even more understandable.

REFERENCES

1. Holland JH. Adaptation in natural and artificial systems. Ann Arbor, Mich: The University of Michigan Press, 1975.
2. Goldberg DE. Genetic algorithms in search, optimization, and machine learning. Reading, Mass: Addison-Wesley, 1989.
3. Mitchell M. An introduction to genetic algorithms. Cambridge, Mass: The MIT Press, 1996.
4. Goldberg DE. Genetic and evolutionary algorithms come of age. Commun Assoc Comput Machinery 1994;37:113-9.
5. Forrest S. Genetic algorithms: principles of natural selection applied to computation. Science 1993;261:872-8.
6. Gongdon CB. A comparison of genetic algorithms and other machine learning systems on a complex classification task from common disease research [Dissertation]. Ann Arbor, Mich: University of Michigan, Department of Computer Science and Engineering, 1995.
7. Laurikkala J, Juhola M. Learning diagnostic rules from a urological database using genetic algorithms. In: Proceedings of The Third Nordic Workshop on Genetic Algorithms and Their Applications, Helsinki, Finnish Artificial Intelligence Society; 1997:233-44.
8. Kentala E, Pyykkö I, Auramo Y, Juhola M. Database for vertigo. Otolaryngol Head Neck Surg 1995;112:383-90.
9. Kentala E, Pyykkö I, Auramo Y, Juhola M. Characteristics of six otologic diseases involving vertigo. Am J Otol 1996; 17:883-92.
10. Michalski R, Kodratoff Y. Research in machine learning. In: Kodratoff Y, Michalski R, eds. Machine learning: an artificial intelligence approach. Vol 3. San Mateo, Calif: Morgan Kaufmann Publishers, 1990:3-30.
11. Grefenstette JJ. Optimization of control parameters for genetic algorithms. IEEE Trans Syst Man Cybern 1986;16:122-8.
12. Schaffer JD, Caruana RA, Eshelman LJ, Das RA. Study of control parameters affecting online performance of genetic algorithms for function optimization. In: Schaffer JD, ed. Proceedings of the Third International Conference on Genetic Algorithms. Palo Alto, Calif: Morgan Kaufmann Publishers, 1989: 51-60.
13. Michalski R. A theory and methodology of inductive learning. In: Michalski R, Carbonell J, Mitchell T, eds. Machine learning: an artificial intelligence approach. Vol 1. Los Altos, Calif: Morgan Kaufmann Publishers, 1983:83-134.
14. Pappas D, Banyas J. A newly recognized etiology of Meniere's syndrome. Acta Otolaryngol Suppl (Stockh) 1991(suppl 485):104-7.
15. Pearson B, Brackmann D. Committee on hearing and equilibrium guidelines for reporting treatment results in Meniere's disease. Otolaryngol Head Neck Surg 1985;93:579-81.
16. Norrving B, Magnusson M, Holtås S. Isolated acute vertigo in the elderly; vestibular or vascular disease? Acta Neurol Scand 1995;91:43-8.
17. Kentala E, Pyykkö I, Auramo Y, Juhola M. Neural networks in neurotologic expert systems. Acta Otolaryngol Suppl (Stockh) 1997(suppl 529):127-9.
18. Forsström J, Dalton K. Artificial neural networks for decision support in clinical medicine. Ann Med 1995;27:509-17.
19. Kentala E, Pyykkö I, Auramo Y, Juhola M. Otoneurological expert system. Ann Otol Rhinol Laryngol 1996;105:654-8.

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COCHLEAR FUNCTION OF GUINEA PIGS WITH EXPERIMENTAL CHRONIC RENAL FAILURE

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This study aimed to evaluate electrophysiologically the cochlear function of guinea pigs that underwent a five-sixths nephrectomy and, additionally, to explore the synergistic action between chronic renal failure (CRF) and noise. Cochlear potentials were recorded at 1, 2, and 3 months postoperatively. Slight changes in compound action potential and cochlear microphonics were seen at 1 month postoperative, while moderate and profound changes were seen at 2 and 3 months. Endocochlear potential showed no significant reduction. The results indicate that CRF may be an etiologic factor for cochlear dysfunction and that the hair cells seem likely to be a main site of the lesion. One-month-postoperative animals were exposed to a broadband noise. In contrast to control animals, the test animals demonstrated no recovery from the decrease in compound action potential and cochlear microphonics that occurred immediately after noise exposure. This suggests a synergistic interaction between CRF and noise.

KEY WORDS — chronic renal failure guinea pigs, electrocochleography, nephrectomy, noise exposure.

INTRODUCTION

The question of whether renal failure is a causative factor in cochlear dysfunction has previously been investigated. Previous human studies involving the assessment of hearing threshold alteration,[1-3] changes in auditory brain stem response (ABR) waveform[4-6] before and after hemodialysis, and morphological studies of the temporal bone[7,8] have been reported. Most of those studies have suggested that renal failure may play an important role in the occurrence of cochlear impairment by various causes. However, it is still difficult to state that the mechanism of cochlear dysfunction in renal failure has been definitively elucidated. Most of the animal studies have been conducted under the acute nephrotoxic condition resulting from bilateral total nephrectomy. Electrophysiological studies in the cochlea have demonstrated a decrease in endocochlear potential (EP) output,[9] and abnormal changes in cochlear microphonics (CM) and action potential (AP).[10] The ABR recordings showed a delay of the first wave[11] and a threshold elevation at lower frequencies.[12] Histochemical investigations showed an increase in osmotic pressure in the serum and perilymph, abnormal changes in the biochemical composition of perilymph,[13] and morphological abnormalities in the stria vascularis.[14] We consider there may be a qualitative difference in the influence on cochlear function of acute nephrotoxicity due to total nephrectomy versus induced chronic renal failure (CRF). Recently, we succeeded in creating guinea pigs with CRF by means of a five-sixths nephrectomy performed in 2 stages and have investigated these CRF animals by electrocochleography (ECochG).[15] This study aimed to evaluate electrophysiologically the cochlear function of guinea pigs that underwent five-sixths nephrectomy and, additionally, to explore the synergistic effects of noise exposure on the cochleas of these CRF animals.

MATERIALS AND METHODS

Healthy Hartley guinea pigs with a normal Preyer's reflex, weighing 250 to 300 g, were used as subjects in this study. All procedures in this experiment were completed in accordance with St Marianna University human and animal investigation committee policies.

CREATION OF CHRONIC RENAL FAILURE

The CRF was created by five-sixths nephrectomy performed separately in 2 stages. General anesthesia was achieved with intraperitoneally administered pentobarbital sodium (Nembutal, 30 mg/kg), and local anesthesia with 1% Xylocaine. One kidney was exposed through the retroabdominal cavity, and two thirds of the kidney was excised. One week later, the contralateral kidney was totally removed by a similar surgical procedure. The development of renal failure was confirmed by detecting a decrease in urine output, an increase in urine protein, and the

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COMPARISON OF BLOOD UREA NITROGEN AND SERUM CREATININE IN NEPHRECTOMIZED ANIMALS VERSUS CONTROLS

		Months After Five-Sixths Nephrectomy		
	Controls (n = 5)	*1 (n = 7)*	*2 (n = 11)*	*3 (n = 8)*
Serum creatinine	0.4 ± 0.1	1.2 ± 0.2	1.2 ± 0.6	1.1 ± 0.2
Blood urea nitrogen	24 ± 3.4	68 ± 6.0	57 ± 2.3	54 ± 9.9

Data are milligrams per deciliter (mean ± SD). Both values were abnormally enhanced 1 month after operation. However, no significant difference was observed between 1, 2, and 3 months postoperatively.

abnormal elevation of serum creatinine and blood urea nitrogen (BUN) levels. Also, the remaining kidney was histologically examined afterward in some animals.

ELECTROPHYSIOLOGICAL MEASUREMENTS

Three experimental groups, consisting of animals with 1, 2, and 3 months of survival after the 2-stage operation, were prepared for ECochG measurements. The noise exposure experiment was carried out at 1 month postoperatively; at this point, cochlear impairments were found to be slight.

Compound Action Potential and Cochlear Microphonics. The guinea pigs underwent tracheotomy under general anesthesia gained by intraperitoneal injection of Nembutal (30 mg/kg) and were artificially ventilated after intramuscular injection of suxamethonium chloride (Relaxin, 50 mg/kg) to inhibit body movements. Then, an active electrode with a silver ball tip was placed on the round window membrane, which was made fully visible by the ventrolateral surgical approach using a microscope. A reference needle electrode and a ground electrode were inserted into the neck muscle and retroauricular muscle, respectively. The elicited electrical responses were amplified by a biological amplifier (Nihon Koden AVB-11), the bandpass filter of which was set at a frequency from 0.5 to 10 kHz, and then sent to a signal processor (Nihon Koden QC-111J) to be averaged 100 times. The threshold of compound action potential (CAP), CM, and latency of CAP were measured in this study. The CAP threshold was defined as the lowest intensity capable of eliciting distinctly visible potentials in the averaged recording, and the CAP latency as the time from the onset of CM to CAP (N1) peak. Clicks having a maximum power spectrum at 4 kHz were used as sound stimuli to record CAP. Tone bursts of frequencies from 2 to 10 kHz with a rise and fall time of 0.5 milliseconds (ms) and a duration of 8 ms were used to record CM. The interstimulus interval (ISI) for click stimulation was 120 ms, and the ISI for tone burst stimulation was 30 ms. These sound stimuli were delivered through a loudspeaker (JBL 2450H) placed at a distance of 40 cm from the tested ear. Stimulus levels were calibrated by the substitution method. The stimulus sound was picked up with a condenser microphone (Rion UC-30) placed at the approximate site of the tympanic membrane. The output of a condenser microphone was sent to a sound level meter (Rion NA-51) and an oscilloscope (Iwatsu DS-8605) to measure the sound waveform and pressure. Since stimuli of very short duration were used, sound pressure was measured in decibels peak equivalent sound pressure level (SPL).

Endocochlear Potential. A glass microelectrode with a tip diameter of about 2 μm, filled with 3-mol/L potassium chloride solution, was introduced into the scala media through the round window membrane by means of a micromanipulator (Narisige M-3333). The elicited potential was fed into a microelectrode amplifier (Nihon Koden MEZ-7200), and EP was measured in millivolts.

NOISE EXPOSURE EXPERIMENT

The influence of noise exposure on the cochlear function of CRF animals was examined in comparison with control animals. One-month-postoperative guinea pigs were used as subjects. The tested ear was exposed to broadband noise (bandwidth 50 to 20,000 Hz) with an intensity of 105 dB SPL for 30 minutes through a loudspeaker (Altec 807-8) located 40 cm distant. Electrophysiological measurements were carried out immediately after the exposure to noise and 1, 2, 3, 4, 5, and 6 hours later. At the end of the test session, a blood sample was drawn to examine the BUN and serum creatinine levels on an autoanalyzer (Hitachi 736). In all experiments, guinea pigs with a normal Preyer's reflex and without any treatment were used as control animals. For statistical analysis, *t*-tests for both paired and unpaired samples were used.

RESULTS

CHANGES IN BUN AND SERUM CREATININE

The BUN and creatinine levels in each group at 1, 2, and 3 months after the five-sixths nephrectomy were compared with those of controls (see Table). Both levels in the postoperative groups were significantly elevated compared to those in controls ($p < .01$). However, no significant difference in levels between 1- and 3-month groups was found.

CHANGES IN COCHLEAR POTENTIALS

Compound Action Potential. Although changes in the threshold and latency of 1-month animals were

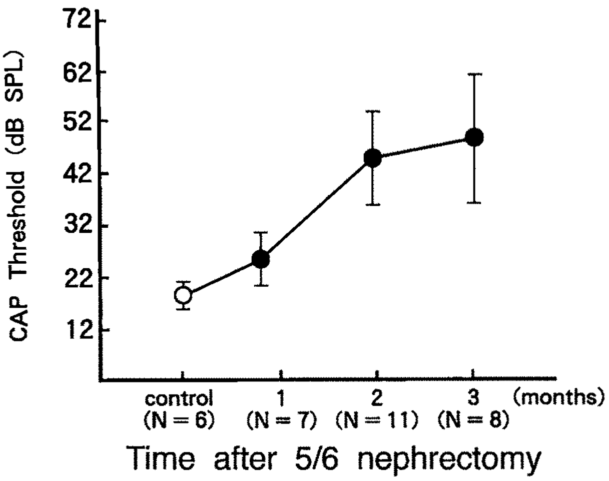

Fig 1. Postoperative change in compound action potential (CAP) thresholds (±1 SD). Threshold elevation was slight at 1 month, while significant changes from preoperative levels were observed for 2- and 3-month groups. SPL — sound pressure level.

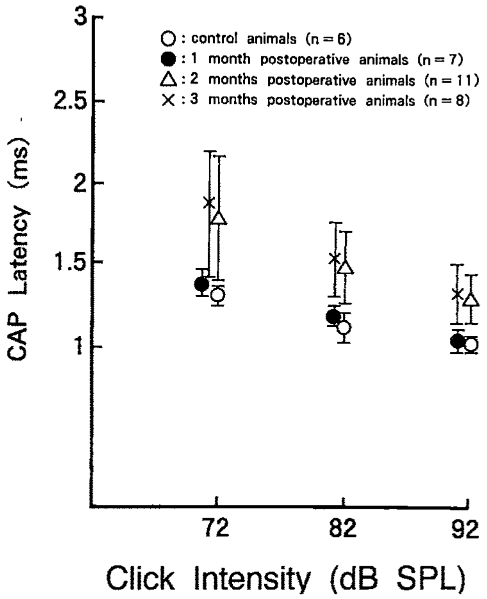

Fig 2. Postoperative change in CAP latency (±1 SD). Similar to threshold curves, distinct increase in latency was seen in 2- and 3-month groups, whereas no significant difference between these groups was found.

mild, those of 2- and 3-month animals were markedly changed. Figure 1 shows the threshold change in relation to postoperative time (months). At 1 month, only a slight threshold elevation of about 7.5 dB compared to controls was seen, while at 2 and 3 months, pronounced average elevations of 27 and 31 dB as compared to control levels were evident. At 3 months, a large spread in threshold values among animals, ranging from 37 to 67 dB, was observed. No significant difference in threshold between the 2-month and 3-month groups was found. However, thresholds in these 2 groups were significantly elevated as compared with controls ($p < .01$). The CAP latency was evaluated in the same way as the threshold (Fig 2). One-month animals presented only a small change in latency, showing no significant difference compared to controls. The 2- and 3-month groups did exhibit a significant increase in latency ($p < .01$) when compared to controls, but no significant difference when compared to each other. Changes in latency with intensity of stimulation were relatively small in all groups, presumably because high intensities of stimulation (72 to 92 dB) were used, as required by the observed threshold shift following nephrectomy. The amplitudes of CAP recorded from the 2- and 3-month groups were generally reduced, and a loss or decrease of the L-component of the input-output relation curve (which consists of an H-component and an L-component) was found in many cases, although there was a great variability in amplitude values across animals in the 2- and 3-month groups. Such intersubject variability might be ascribed to overresection or inadequate resection of the kidney. Figure 3 demonstrates representative ECochG waveforms obtained from postoperative and control animals. Although a 1-month animal showed only a slight change in CAP, a 3-month animal distinctly demonstrated a threshold elevation, a decrease in amplitude, and an increase in the latency of re-

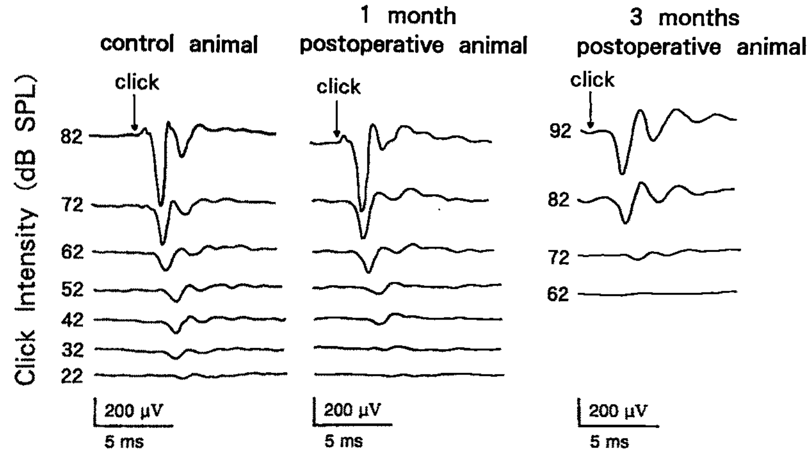

Fig 3. Representative CAP waveforms from postoperative animals compared to control CAP. Only slight change was shown in 1-month animal, while marked threshold elevation, latency prolongation, and amplitude reduction were observed in 3-month animal.

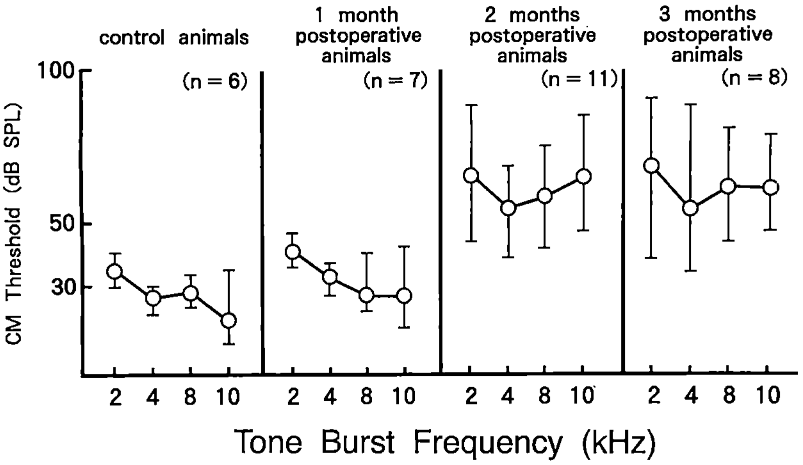

Fig 4. Postoperative change in cochlear microphonic (CM) thresholds (± maximum and minimum). Similar to CAP threshold curves, slight elevation was indicated in 1-month group, while 2- and 3-month postoperative groups presented marked elevation with no constant relationship between threshold change and stimulus frequency.

sponse, as shown in the right waveform array. A number of 2- and 3-month animals (59% of cases) exhibited a decrease and/or loss of the L-component, as demonstrated in this case (right waveforms in the Figure).

Cochlear Microphonics. Figure 4 depicts the CM threshold changes in relation to tone burst frequencies in 1-, 2-, and 3-month animals. In this Figure, the variance indicated by vertical bars in the curve is expressed by maximum and minimum values instead of standard variation (SD), to take into account the great variation across animals. The 2- and 3-month groups distinctly showed threshold elevation to whole tone burst frequencies from 2 to 10 kHz relative to controls ($p < .01$). No apparent constant relationship between threshold elevation and stimulus frequency could be found.

Endocochlear Potential. The EP outputs obtained from 1-month (4 animals) and 3-month animals (3 animals), all of which demonstrated abnormal changes in CAP and CM, are presented in Fig 5. To take the small number of animals into account, the degree of deviation is expressed by maximum and minimum values in this Figure. No significant decrease in EP of postoperative animals was seen as compared to controls, regardless of abnormal findings of CM and CAP.

EFFECT OF BROADBAND NOISE ON CAP AND CM RECORDED AT ONE MONTH

Compound Action Potential Threshold. Figure 6 presents curves showing alterations of thresholds versus time (hours) following noise exposure in nephrectomized animals and controls. The curves are composed of mean values ±1 SD. The control animals exhibited a marked threshold elevation of 29.0 ± 4.4 dB immediately after noise exposure, and thereafter the threshold level declined gradually with time. Six hours after noise exposure, an elevation of 18.6 ± 5.2 dB was shown. This elevated level was not statistically significant compared to the preexposure level (14.5 ± 1.9 dB). On the other hand, the nephrec-

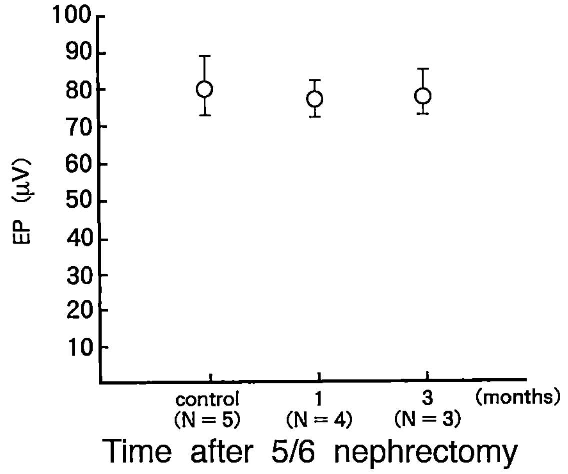

Fig 5. Postoperative change in endocochlear potential (EP; ± maximum and minimum). No significant decrease in EP of postoperative animals was shown, as compared to controls.

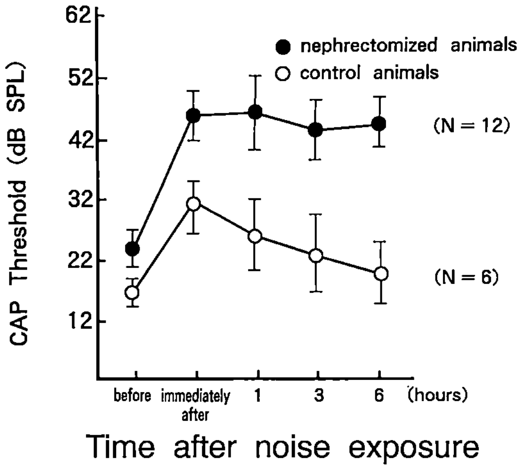

Fig 6. Change in CAP (click stimulus) threshold with time after noise exposure (±1 SD). No recovery of thresholds in nephrectomized animals to preexposure level was demonstrated, even at 6 hours postexposure.

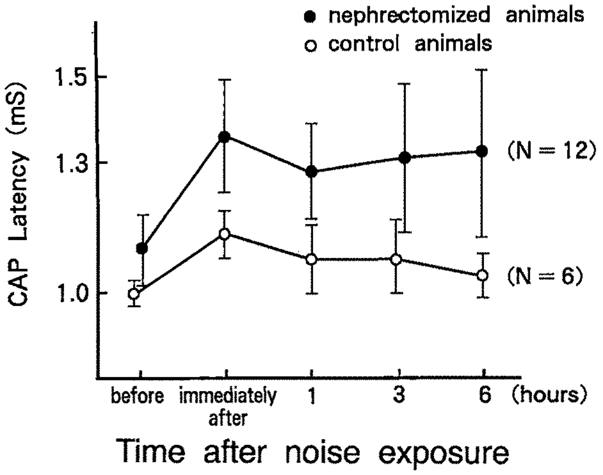

Fig 7. Change in CAP (click stimulus at intensity of 92 dB SPL) latency with time after noise exposure (±1 SD). Similar to threshold, no recovery at 6 hours after exposure was observed in nephrectomized animals.

tomized animals' curve showed a threshold elevation of 43.1 ± 4.8 dB immediately after noise exposure, with no decline in levels over time. Six hours after exposure, there was a threshold elevation of 42.2 ± 4.3 dB, which was significantly higher than the preexposure level (22.4 ± 3.0 dB; $p < .0001$).

Compound Action Potential Latency. A study of the CAP latency similar to that of the CAP threshold is demonstrated in Fig 7. The stimulus was a click of 92 dB SPL. The control curve showed an increase in latency (1.14 ± 0.05 ms) immediately after noise exposure (like the CAP threshold), followed by a gradual decrease with time until 6 hours later, when the latency had recovered (1.05 ± 0.05 ms) almost to the preexposure value (1.00 ± 0.03 ms). On the other hand, the nephrectomized animals' curve showed an increased latency of 1.36 ± 0.14 ms immediately after noise exposure, and 6 hours later, an increased value of 1.32 ± 0.20 ms still persisted. This latency value was significantly prolonged compared to that preexposure (1.10 ± 0.08 ms; $p < .01$).

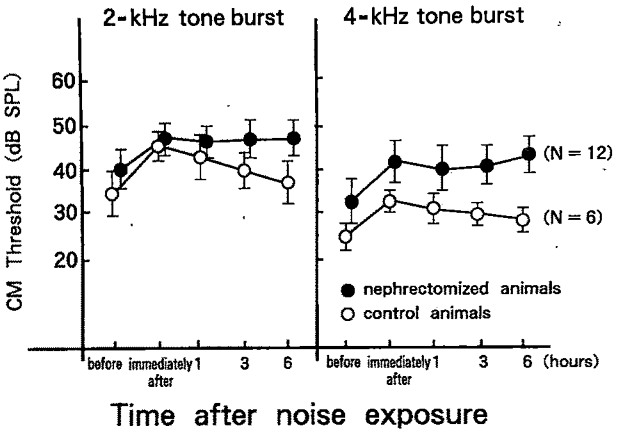

Fig 8. Change in CM thresholds with time after noise exposure (±1 SD). Control animals showed no significant threshold elevation even at 6 hours after exposure, while nephrectomized animals exhibited significantly increased thresholds 6 hours after exposure to both tone bursts.

Cochlear Microphonic Threshold. Figure 8 demonstrates the alteration in CM thresholds to 2- and 4-kHz tone bursts as a function of time (hours) after noise exposure in comparison with controls. The thresholds in control animals were temporarily elevated immediately after noise exposure, like the CAP threshold, and tended to decline in time. Six hours after noise exposure, the thresholds to both tone bursts did not remain significantly elevated, as compared to the preexposure levels. In contrast, the thresholds of nephrectomized animals to 2- and 4-kHz tone bursts at 6 hours after noise exposure remained elevated: 47.3 ± 4.4 and 42.7 ± 4.5 dB SPL, respectively. These threshold levels were significantly higher than the preexposure levels: 39.6 ± 4.4 and 33.8 ± 4.7 dB SPL ($p < .05$).

Summating Potential Polarity. The change in the AP–summating potential (SP) complex waveform to a 10-kHz tone burst after noise exposure is displayed in Fig 9. The left and right response arrays in this Figure were obtained from a control animal and a

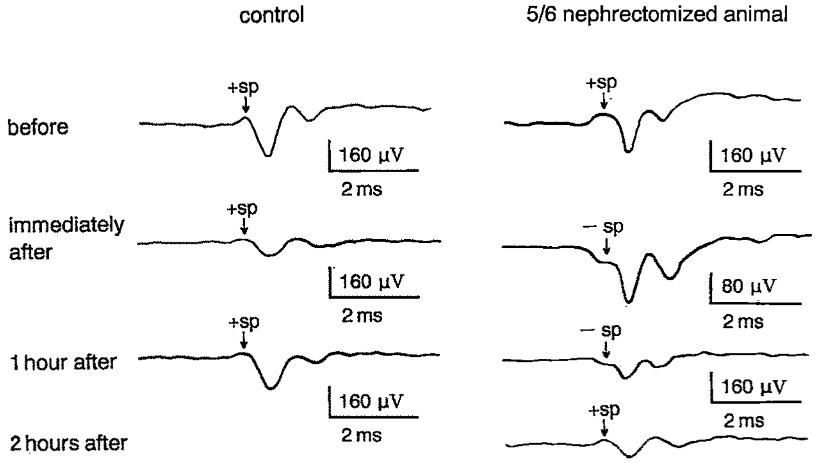

Fig 9. Effect of noise exposure on electrocochleography waveform to 10-kHz tone burst. Example indicates reversal of summating potential (SP) polarity by noise exposure. In 4 of 12 nephrectomized animals, reversal of polarity from +SP to –SP, as seen in right side of Figure, was recorded immediately and 1 hour after noise exposure.

nephrectomized animal, respectively. In the nephrectomized guinea pig, the reversal of SP polarity from positive to negative was recorded immediately after and 1 hour after noise exposure, distinctly shown in the middle and lower waveforms in the right side. Two hours later, the SP polarity again returned to positive. Such a change in polarity was observed in 4 of 12 nephrectomized animals (33%), but was never recorded in control animals.

Endocochlear Potential Output Voltage. The EP in millivolts was measured in 3 nephrectomized guinea pigs 6 hours after noise exposure. The EP voltages of these 3 guinea pigs (74, 77, and 80 mV) showed no significant reduction when compared with the normal control value (79.8 ± 7.5 mV).

DISCUSSION

A number of experimental studies examining the influence of renal failure on cochlear function have been conducted in the past. These studies were performed on rats and relied on acute total bilateral nephrectomy. Urquiza and Morell[9] found a decrease in EP in rats with bilateral nephrectomy and considered that the decrease in Na-K adenosinetriphosphatase (ATPase) activity may be responsible for this EP change. In a later study,[13] they demonstrated abnormal changes in osmotic and biochemical properties of the perilymph and suggested that these disturbances in perilymph may induce a failure of labyrinthine fluid microhomeostasis, resulting in cochlear disturbances. Adler et al,[16] using a similar procedure with guinea pigs as the experimental animal, found that the intracochlear Na-K ATPase system is inhibited by a toxic metabolite produced by or accumulated in renal insufficiency. Urquiza and Ciges[14] carried out a detailed ultrastructural investigation on the cochlear disturbances caused by acute renal insufficiency using Mongolian gerbils as subjects and found characteristic morphological changes in the stria vascularis of absorptive secretory areas. With respect to electrophysiological investigations, Anniko and Hellstrom[12] recorded the ABR from rats with acute uremia following bilateral nephrectomy. They found that initially, low-frequency threshold and latency were affected, but over time, high-frequency responses became impaired as well. They assumed that the functional disturbances may start at the cochlear apex, where the volume of the stria vascularis is small, and later extend toward the base of the cochlea, where the volume of the stria vascularis is increased. They assumed that changes in the response patterns were caused by a disturbed homeostasis of the inner ear fluids, leading ultimately to hair cell damage. Many experimental results in the past have suggested that the site of functional impairments in the cochlea following renal failure may be the stria vascularis.[8,14,17]

On the other hand, Ikeda et al[10] showed the inhibition of CM and CAP within the intact EP using guinea pigs with experimentally induced renal failure and considered the sensory hair cells most probable as the involvement site of cochlear dysfunction. Shvili et al[11] and Lucien et al[6] found prolongation of wave I and wave V of the ABR, suggesting involvement of the eighth nerve, in rats with chronic renal failure and a patient suffering from uremic insufficiency, respectively. We suppose that there is a qualitative difference in the effect of renal failure on the cochlear function between the above-described acute uremia and our experimentally induced CRF.

In an attempt to evaluate the functional properties of the cochlea with CRF induced by five-sixths nephrectomy, Shvili et al[11] studied the ABR in rats 3 months after surgery. They found that the wave I latency was delayed despite a normal I-V interpeak interval, and suggested that a disturbance of neural conduction along the acoustic nerve or cochlear changes might arise early in CRF. Merck et al[17] reported morphological changes in the stria vascularis in rats with the same surgical technique. Kenmochi et al[15] showed abnormal changes in the CM and CAP, using the same nephrectomizing procedure in guinea pigs, and assumed the occurrence of cochlear hair cell damage at 2 months after operation. The present study also demonstrated an apparent decrease in the amplitudes of CM and CAP, and a prolongation of CAP latency, at 2 and 3 months postoperatively. However, it was serologically apparent that renal failure arose as early as 1 month after surgery, although with smaller changes in the CM and CAP. The 1-month ECochG changes were found to be still slight in degree, although develpment of renal failure was serologically evident.

This discrepancy is still unresolved. A comparison of the data of our method with those of acute total nephrectomy should be elaborated. Because the 3-month ECochG results showed a great variability across animals, the average values of the threshold and latency revealed little difference from those of the 2-month animals. The 3-month ECochG changes became pronounced in conjunction with renal failure aggravation. However, some animals showed moderate abnormalities of ECochG because of inadequate nephrectomy. Accordingly, the ECochG results in this group were highly variable across animals.

As for the EP, a small number of animals was studied, and no significant decrease in EP output was seen, despite the abnormal postoperative changes in CM and CAP as compared with control animals. Needless to say, a study using a larger number of animals is essential for thorough evaluation of the EP. The lack of significant EP changes indicates that cochlear hair cells, rather than the stria vascularis, are likely responsible for the generation of cochlear dysfunction in our experiment.

We suggest that the cause of the cochlear dysfunction resulting from CRF is different from that resulting from acute uremic insufficiency. We suggest that lesions within the cochlea, observed in our physiological measurements, might develop over time following CRF to affect the cochlear hair cells. Regarding the effect of noise exposure on the cochlear potentials, we observed that sound levels not expected to cause serious cochlear injury produced a significantly abnormal ECochG outcome in CRF guinea pigs. Whether or not the superimposition of more than 2 risk factors for cochlear impairments can give rise to a synergistic effect presents an interesting and important clinical question. Raynor et al[18] suggested that noise exposure in animals with diabetes mellitus induced by streptozotocin could accelerate the vulnerability of cochlear hair cells. Pillsbury[19] reported that the vulnerability of the cochlea in hypertensive rats with hyperlipidemic blood was increased by presentation of noise. Regarding the interaction between noise and other agents, combined effects have been investigated for noise and the following: salicylates,[20] gentamicin and other aminoglycosides,[21] cisplatin,[22] and loop-inhibiting diuretics.[23] An evident synergistic action was ascertained between noise and the above agents except for salicylates and loop diuretics. Further, it has been suggested that cochlear impairments (hair cell damage) may even occur from the interaction of agents present in subototoxic concentrations and noise exposure of less injurious intensity.[21,22,24] To the authors' knowledge, there have been no studies on the synergistic effects of noise and renal failure.

In the present study, five-sixths–nephrectomized guinea pigs demonstrated the combined effect of CRF and noise exposure, and the effect was an exacerbation of cochlear dysfunction. An irreversible threshold elevation of CM and CAP and a prolongation of CAP latency were observed in the guinea pigs after noise exposure. Additionally, a reversal of SP polarity and maintenance of EP output within normal limits were recorded in a small number (3) of animals.

To our regret, this study was lacking in histopathology. However, if we take the above-described changes in cochlear potentials into account, hair cell dysfunction seems most likely responsible for cochlear impairments provoked as a result of a synergistic action between CRF and noise exposure. We have assumed that the production of nephrotoxic metabolites in five-sixths–nephrectomized guinea pigs can cause cochlear impairment.[15] Sound overstimulation may accelerate the activity of transduction channels in cochlear hair cells, as suggested by Aran et al,[23] and consequently could facilitate entry of the molecules of toxic products into the hair cells, resulting in augmentation of the cochlear impairment due to a synergistic interaction. Additionally, it also is supposed that metabolic disturbances in the cochlea of CRF animals provided by intense noise may fail to recover within the observed time of our measurement.

At the present time, a large number of patients with CRF live relatively normal lives due to hemodialysis. However, the opportunity for them to encounter chemical, physical, and other harmful ototoxic risk factors must surely be increased. About 40% of patients with CRF undergoing hemodialysis are known to have hearing loss.[1,7] On the other hand, there is a view that clinically significant sensorineural hearing loss is not associated with nongenetic kidney disease.[3] Regarding the occurrence of hearing impairment, the mechanism of cochlear dysfunction in chronic renal disturbances has not been fully resolved. The influence of harmful ototoxic risk factors as described above on cochlear function in CRF subjects should be accurately investigated in the future. Finally, we maintain that the mechanism of cochlear dysfunction in patients with CRF must be actively investigated, especially taking into consideration its social importance.

REFERENCES

1. Johnson DW, Mathog RH. Hearing function and chronic renal failure. Ann Otol Rhinol Laryngol 1976;85:43-9.

2. Gatland D, Tucker B, Chalstrey S, Keene M, Baker L. Hearing loss in chronic renal failure. Hearing threshold changes following haemodialysis. J R Soc Med 1991;84:587-9.

3. Klinger AB, Solangi KB, Ventry IM, Goodman AI, Weseley SA. Hearing impairment associated with chronic renal failure. Laryngoscope 1981;91:583-92.

4. Kustel M, Buki B, Gyimesi J, Mako T, Komora V, Ribari O. Auditory brainstem potentials in uraemia. ORL 1993;55:89-92.

5. Magliulo G, Gagliardi M, Ralli G, Persichetti S, Muscatello M. BSER audiometry in haemodialysis patients. Clin Otolaryngol 1987;12:249-54.

6. Lucien JC, Anteunis MA, Jacob MV, Mooy MD. Hearing loss in a uraemic patient: indications of involvement of the VIII nerve. J Laryngol Otol 1987;101:492-6.

7. Bergstrom LV, Thompson P, Sando I, Wood RP. Renal

disease. Its pathology, treatment and effects on the ear. Arch Otolaryngol 1980;106:567-72.

8. Oda M, Preciado MC, Quick CA, Paparella MM. Labyrinthine pathology of chronic renal failure patients treated with hemodialysis and kidney transplantation. Laryngoscope 1974; 84:1489-506.

9. Urquiza R, Morell M. El potentiol endocochlear en la insufficiencia renal experimental. Rev Esp Fisiol 1985;41:407-10.

10. Ikeda K, Kusakari J, Arakawa E, Ohyama K, Inamura N, Kawamoto K. Cochlear potentials of guinea pigs with experimentally induced renal failure. Acta Otolaryngol Suppl (Stockh) 1987(suppl 435):40-5.

11. Shvili Y, Gafter V, Zohar Y, Talmi P, Levi J. Brainstem auditory evoked responses in rats with experimental chronic renal failure. Clin Sci 1989;76:415-7.

12. Anniko M, Hellstrom S. Functional changes in the inner ear in renal insufficiency. An experimental study in the rat. ORL J Otorhinolaryngol Relat Spec 1989;51:103-7.

13. Urquiza R, Morell M. Effects of experimental renal insufficiency on the osmotic pressure and the biochemical composition of perilymph. Acta Otolaryngol (Stockh) 1986;102: 234-8.

14. Urquiza R, Ciges M. Effects of renal insufficiency on the cochlear absorptive-secretory areas. A morphological study in the Mongolian gerbil. Acta Otolaryngol Suppl (Stockh) 1990 (suppl 478):3-15.

15. Kenmochi M, Ohashi T, Ochi K, Kinoshita H, Takeyama I. ECoG study in animals with experimental chronic renal insufficiency. In: Hoemann D, ed. Handbook of ECoG, CAE and intraoperative monitoring. New York, NY: Kugler Publications, 1993:45-8.

16. Adler D, Fiehn W, Ritz E. Inhibition of Na,K-stimulated ATPase in the cochlea of the guinea pig. Acta Otolaryngol (Stockh) 1980;90:55-60.

17. Merck W, Seyler H, Curten I. Ultrastrukturelle Veranderung an der Stria vascularis und dem Ligamentum spirale bei chronische-uramische Ratten. Arch Otorhinolaryngol 1976; 214:63-70.

18. Raynor EM, Vincent N, Carrasco N, Prazma J, Pillsbury HC. An assessment of cochlear hair cell loss in insulin-dependent diabetes mellitus diabetic and noise exposed rats. Arch Otolaryngol Head Neck Surg 1995;121:452-6.

19. Pillsbury HC. Hypertension, hyperlipoproteinemia, chronic noise exposure: is there synergism in cochlear pathology? Laryngoscope 1986;96:1112-38.

20. Carson SS, Prazma J, Pulver SH, Anderson T. Combined effects of aspirin and noise in causing permanent hearing loss. Arch Otolaryngol Head Neck Surg 1989;115:1070-5.

21. Alles RM, Pye A. Cochlear damage in guinea pigs following contralateral sound stimulation with and without gentamycin. Br J Audiol 1993;27:183-93.

22. Boettcher FA, Gratton MA, Bancroft BR, Spongr V. Interaction of noise and other agents: recent advances. In: Dancer AL, Henderson D, Salvi RJ, Hamernick S, eds. Handbook of noise-induced hearing loss. St Louis, Mo: Mosby–Year Book, 1992:175-87.

23. Aran JM, Hayashida T, Erre JP, et al. Noise, aminoglycosides and diuretics. In: Dancer AL, Henderson D, Salvi RJ, Hamernick S, eds. Handbook of noise-induced hearing loss. St Louis, Mo: Mosby–Year Book, 1992:188-95.

24. Marques DM, Clark CS, Hawkins JE Jr. Potentiation of cochlear injury by noise and ototoxic antibiotics in guinea pigs [Abstract]. J Acoust Soc Am 1975;57(suppl 1):60.

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DISTRIBUTION OF PSAMMOMA BODIES IN THE INTERNAL AUDITORY CANAL AND ITS EXTENDED AREAS IN THE HUMAN TEMPORAL BONE

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The internal auditory canal (IAC) and its extended areas of 27 normal human temporal bone specimens were investigated histologically for the distribution of psammoma bodies. A total of 145 ± 25 (mean ± SE) psammoma bodies were counted in series of every tenth 30-µm-thick section. Psammoma bodies were observed in the IAC and around the labyrinthine portion of the facial nerve (FN), the geniculate ganglion of the FN, and the posterior ampullary nerve in the singular canal. The number of psammoma bodies increases with age. We believe that psammoma bodies are a normal finding of aging in the IAC. The compression of the FN by psammoma bodies in the labyrinthine portion of the facial canal and the distribution of numerous psammoma bodies surrounding the posterior ampullary nerve in the narrow singular canal raise the questions of the involvement of psammoma bodies in the FN and in vestibular dysfunction and the presence of psammoma bodies in the subarachnoid space.

KEY WORDS — internal auditory canal, psammoma body.

INTRODUCTION

Routine histopathologic examinations of temporal bone specimens in our laboratory often reveals psammoma bodies in the internal auditory canal, most commonly from aged subjects. Psammoma bodies are roughly spherical, laminar, and calcified structures that are commonly observed in meningiomas,[1-3] papillary carcinomas of the thyroid,[4,5] and serous carcinomas of the ovary.[6] Psammoma bodies are observed occasionally in conditions such as toxic goiter,[7] brain tumors other than meningiomas,[8] papillary adenocarcinoma of the lung,[9] papillary adenocarcinoma of the endometrium,[10] and papillary cystadenoma of the epididymis.[11] They are also observed in normal meninges, choroid plexus, and pineal gland of aged individuals.[2,4,12-16] The prevalence of psammoma bodies in the internal auditory canal has never been examined. This study was performed in 27 normal human temporal bone specimens, to test the hypothesis that the presence of psammoma bodies in the internal auditory canal is an age-related finding that has the potential to produce facio-acoustico-vestibular disturbances.

MATERIALS AND METHODS

This study examined histologic sections from 27 normal temporal bone specimens obtained from 27 individuals (16 males, 11 females; ages 1 week to 83 years) without a history of congenital ear anomalies or brain tumors. Ten specimens were chosen at random from 700 available specimens from individuals 1 week to 19 years old at death; 8 specimens were chosen from 200 specimens from individuals 20 to 59 years old at death; and 9 specimens were chosen from 250 specimens from individuals more than 60 years old at death. Twenty specimens were from the left side and 7 specimens were from the right side. All temporal bones were harvested at autopsy, fixed in 10% formalin solution, decalcified in 5% trichloroacetic acid solution, dehydrated in graded solutions of alcohol, and embedded in celloidin, as described by Sando et al.[17] The specimens were cut in the horizontal plane at a thickness of 30 µm. Every 10th section was stained with hematoxylin and eosin and mounted on a glass slide. Sections through the internal auditory canal, the facial canal, the singular canal, and the lamina cribrosa were used for this study. The total number of psammoma bodies was determined in soft tissue surrounding the facial nerve (FN), the cochlear nerve (CN), the superior division of the vestibular nerve (SVN), and the inferior division of the vestibular nerve (IVN). The area surrounding the FN in the internal auditory canal was divided into a medial half and a lateral half by drawing a line through the midpoints between the porus and the medial margin of Bill's bar (Fig 1A). The areas surrounding the other 3 nerves in the internal

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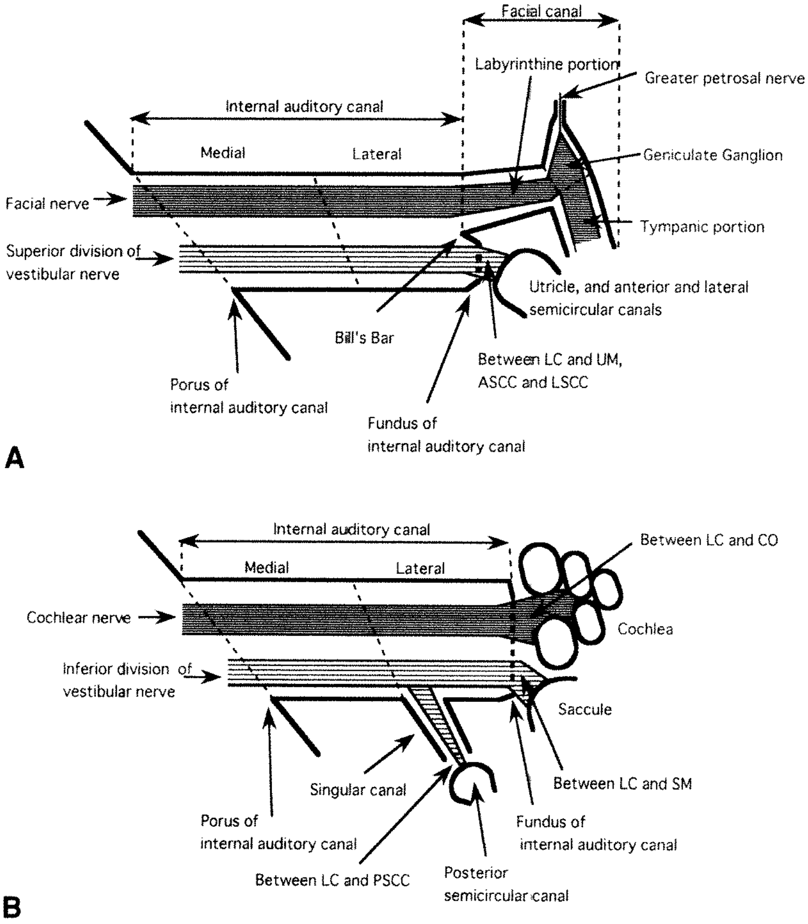
Facial canal
Labyrinthine portion
Greater petrosal nerve
Internal auditory canal
Medial
Lateral
Geniculate Ganglion
Facial nerve
Tympanic portion
Superior division of vestibular nerve
Utricle, and anterior and lateral semicircular canals
Bill's Bar
Between LC and UM, ASCC and LSCC
Porus of internal auditory canal
Fundus of internal auditory canal
A
Internal auditory canal
Between LC and CO
Medial
Lateral
Cochlear nerve
Cochlea
Inferior division of vestibular nerve
Saccule
Singular canal
Between LC and SM
Porus of internal auditory canal
Fundus of internal auditory canal
Between LC and PSCC
Posterior semicircular canal
B

Fig 1. Schema of right internal auditory canal and its extended areas (view from top). A) Superior portion. B) Inferior portion. ASCC — anterior semicircular canal crista, CO — Corti's organ, LC — lamina cribrosa, LSCC — lateral semicircular canal crista, PSCC — posterior semicircular canal crista, SM — saccular macula, UM — utricular macula.

auditory canal were also divided into medial and lateral halves by drawing lines through the midpoints between the porus and the fundus (Fig 1). The numbers of psammoma bodies were tabulated separately for the labyrinthine, geniculate ganglion, and tympanic portions of the facial canal, the singular canal, the region between the lamina cribrosa and Corti's organ, the utricle, the saccule, and the 3 (anterior, lateral, and posterior) semicircular canal cristae.

RESULTS

Psammoma bodies were round to ovoid, laminar, basophilic structures (Fig 2) ranging from 10 to 100 μm in diameter. They were present in 26 of the 27 normal human temporal bone specimens. Psammoma bodies were absent only in case 1, a neonate (see Table). Psammoma bodies were observed around all 4 nerves (FN, CN, SVN, IVN) in the internal auditory canal. In addition, they were found around the labyrinthine portion and the geniculate ganglion of the FN in the facial canal and also around the posterior ampullary nerve (part of the IVN) in the singular canal (Figs 3 and 4). By contrast, psammoma bodies were not found around the tympanic portion of the FN, around the SVN and IVN between the lamina cribrosa and the vestibular end organs, or around the CN between the lamina cribrosa in the cochlear modiolus and the organ of Corti.

We observed 15 ± 3 (mean ± SE) psammoma bodies around the FN in the internal auditory canal and labyrinthine portion as well as the geniculate ganglion portion of the facial canal, 47 ± 8 around the CN, 23 ± 4 around the SVN in the internal auditory canal, and 60 ± 12 around the IVN in the internal auditory canal and the singular canal. Figure 5 shows the distribution of psammoma bodies around each nerve in the medial half and the lateral half of the internal auditory canal and its extended areas.

Two-way repeated-measures analysis of variance (factors: nerve [FN, SVN, CN, and IVN] and laterality [medial versus lateral aspect of each nerve]) and least significant difference (LSD) post hoc comparisons were used to test the hypotheses that within the internal auditory canal, 1) psammoma bodies are distributed differentially among nerves and 2) psammoma bodies are distributed preferentially in the lateral aspect of each nerve. Both of the main effects (nerve: $F(3,104) = 10.2$, $p < .01$; laterality: $F(1,104) = 90.9$, $p < .01$) and the interaction effect (nerve × laterality: $F(3,104) = 9.4$, $p < .01$) were significant. Post hoc tests indicated that these effects reflect 3

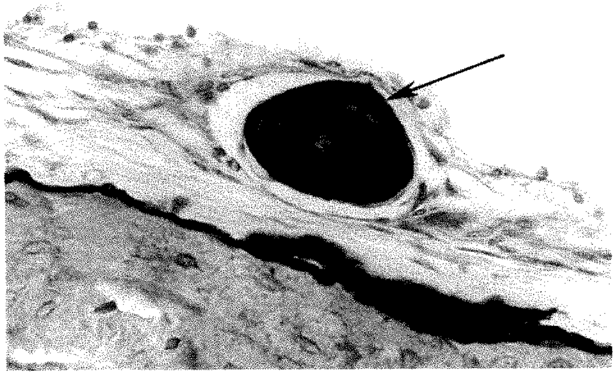

Fig 2. (Case 27) Round and laminar psammoma body (arrow) observed near posterior wall of internal auditory canal (H & E, original ×613).

AGE AND NUMBER OF PSAMMOMA BODIES IN INTERNAL AUDITORY CANAL AND ITS EXTENDED AREAS OF 27 HUMAN TEMPORAL BONES

Case No.	*Age*	*Sex*	*Side*	*No. of Psammoma Bodies*	*Cause of Death*
1	1 wk	M	R	0	Hyaline membrane disease
2	2 y	F	L	11	Tetralogy of Fallot
3	3 y	F	L	43	Liver rupture
4	4 y	M	L	37	Cystic fibrosis
5	6 y	M	L	39	Alveolar rhabdomyosarcoma
6	10 y	M	L	66	Leukemia
7	12 y	M	R	66	Adrenal leukodystrophy
8	14 y	M	L	67	Congenital anti-thrombin disorder
9	18 y	M	L	120	Leukemia
10	18 y	M	L	176	Renal failure
11	21 y	M	L	169	Pulmonary embolism
12	23 y	F	L	38	Suffocation
13	40 y	F	L	119	Coronary artery disease
14	43 y	M	R	158	Thrombocytopenia
15	43 y	F	L	129	Myocardial infarction
16	51 y	F	R	109	Staphylococcal pneumonia
17	56 y	F	L	208	Pharyngeal carcinoma
18	58 y	F	R	104	Pulmonary embolism
19	60 y	M	L	275	Maxillary carcinoma
20	64 y	M	L	136	Tonsil cancer
21	69 y	F	R	251	Parkinson's disease
22	71 y	M	L	256	Leukemia
23	74 y	F	L	89	Lung cancer
24	76 y	M	L	290	Coronary artery disease
25	79 y	M	R	69	Esophageal cancer
26	81 y	F	L	642	Cholangitis
27	83 y	M	L	256	Sudden death (unknown)

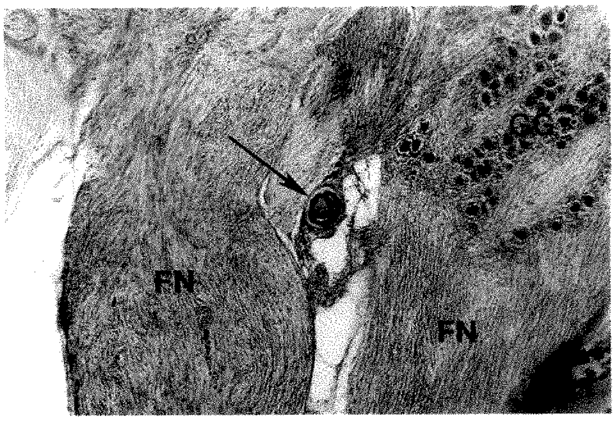

Fig 3. (Case 18) Psammoma body (arrow) around geniculate ganglion (GG; H & E, original ×130). FN — facial nerve.

main findings. First, for each nerve, there was a greater number of psammoma bodies laterally than medially (Fig 6, LSD test, $p < .01$). Second, the number of psammoma bodies in the lateral aspect of the nerves for both the CN and IVN was greater than for either the SVN or FN (Fig 6, LSD test, $F(3,104) = 10.1$, $p < .01$; Fig 7). However, there was no significant difference in the number of psammoma bodies in the lateral aspect of the nerves between the CN and IVN or between the SVN and FN. Finally, there was no significant difference in the number of psammoma bodies in the medial aspect of each nerve ($F(3,104) = 0.5$, not significant).

Linear regression analysis indicated that the number of psammoma bodies increases with age (Pearson's correlation coefficient = .653, $p < .01$, Fig 8). There were no statistically significant differences between males and females, nor between the right side and left side (Student's *t*-test, not significant).

DISCUSSION

This study provides the first quantitative informa-

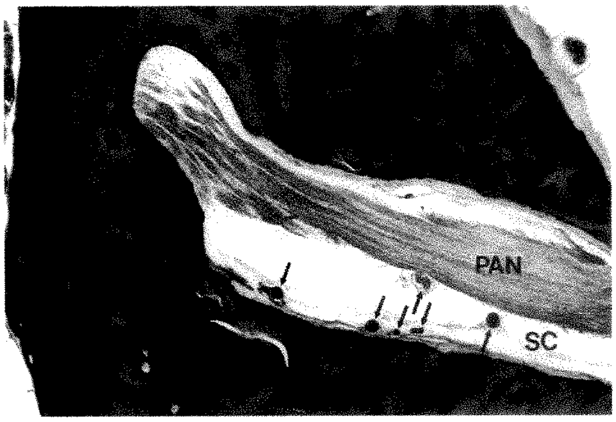

Fig 4. (Case 24) Psammoma bodies (arrows) in singular canal (SC; H & E, original ×58). PAN — posterior ampullary nerve.

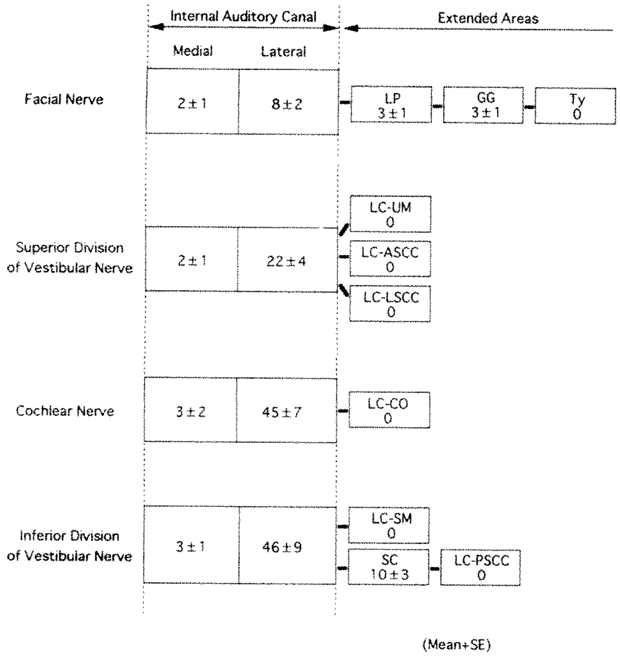

Fig 5. Distribution of psammoma bodies in internal auditory canal and its extended areas. ASCC — anterior semicircular canal crista, CO — Corti's organ, GG — geniculate ganglion, LC — lamina cribrosa, LP — labyrinthine portion, LSCC — lateral semicircular canal crista, PSCC — posterior semicircular canal crista, SC — singular canal, SM — saccular macula, Ty — tympanic portion, UM — utricular macula.

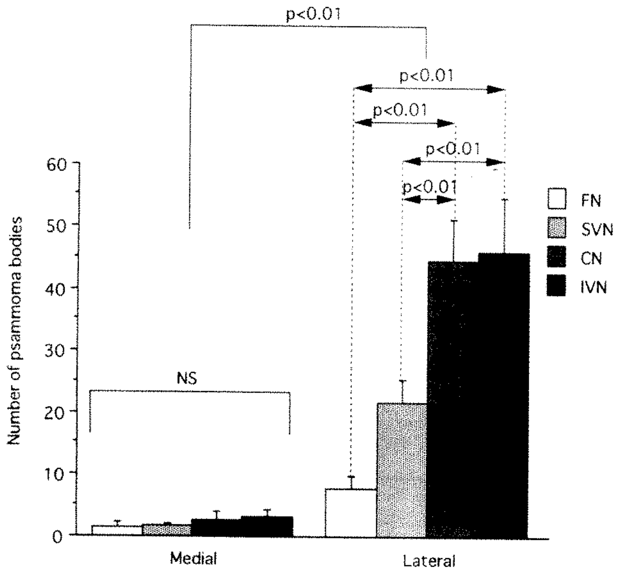

Fig 6. Two-way repeated-measures analysis of variance with factors of nerve (facial nerve [FN], superior division of vestibular nerve [SVN], cochlear nerve [CN], and inferior division of vestibular nerve [IVN]) and laterality (medial versus lateral aspect of each nerve), and least significant difference post hoc comparisons. There was greater number of psammoma bodies laterally than medially for each nerve. Number of psammoma bodies in lateral aspect of nerves for both CN and IVN was greater than for either SVN or FN. There was no significant difference (NS) in number of psammoma bodies in medial aspect of each nerve.

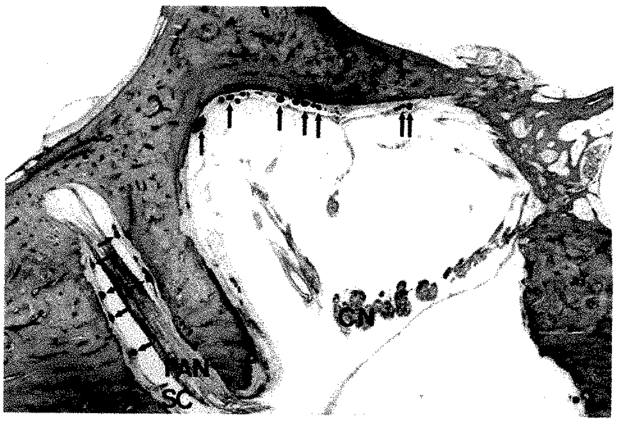

Fig 7. (Case 26) Psammoma bodies (arrows) near cochlear nerve (CN) at fundus of internal auditory canal and also posterior ampullary nerve (PAN) in singular canal (SC; H & E, original ×33).

tion on the distribution of psammoma bodies in the internal auditory, facial, and singular canals. Although psammoma bodies in these areas have not been reported, psammoma bodies in the cranium were reported to be formed by normal arachnoid cells[2,3,15,16] or by neoplastic cells.[1-3,8,13,14] Both normal arachnoid cells and neoplastic arachnoid cells can form whorls[2] and synthesize several collagen subtypes.[18] Psammoma bodies in normal arachnoid or meningiomas generate the whorls and concentric collagen fibers. Extracellular matrix vesicles are also produced by normal or neoplastic arachnoid cells. Calcification occurs in the matrix vesicles, and the mineralization process extends to the neighboring collagen fibers. These calcified masses form psammoma bodies.[14,15,19,20] In our cases, with no brain tumors, psammoma bodies were present in soft tissue surrounding nerves within the internal auditory, facial, and singular canals. Arachnoid cells were identified around some of the psammoma bodies (Fig 2), but were not identified clearly around other psammoma bodies (Fig 9). The areas in which psammoma

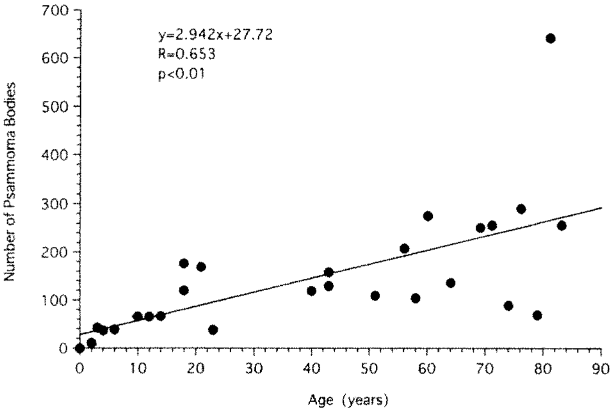

Fig 8. Correlation between number of psammoma bodies in internal auditory canal and its extended areas and ages of 27 individuals (Pearson's correlation coefficient = .653, p < .01).

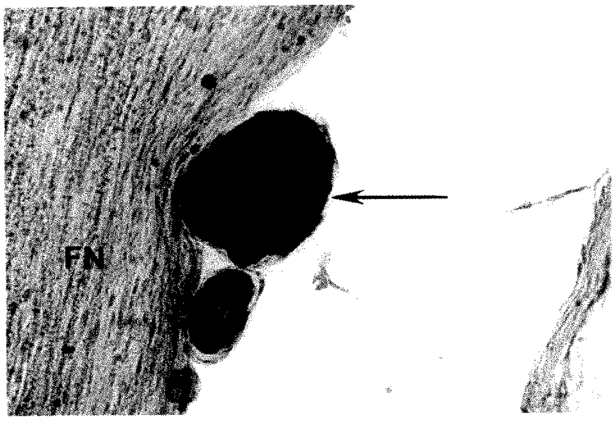

Fig 9. (Case 22) Psammoma body (arrow) compressing surface of facial nerve (FN) in its labyrinthine portion (H & E, original ×514).

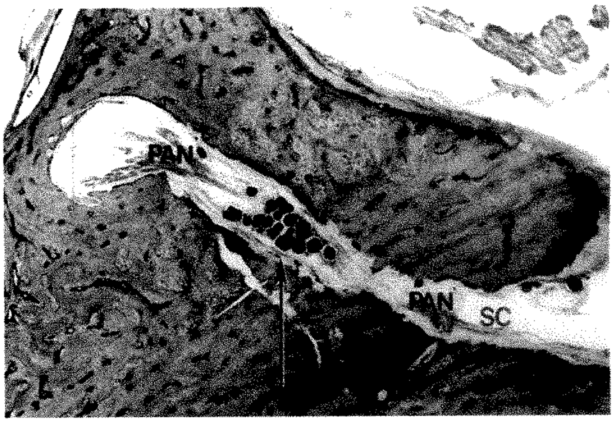

Fig 10. (Case 26) Number of psammoma bodies (arrow) were present along posterior ampullary nerve (PAN; part of inferior division of vestibular nerve) in singular canal (SC; H & E, original ×50).

bodies were present corresponded to the reported extent of the arachnoid cells. Perlman and Lindsay[21] traced the arachnoid cells around the FN to the mouth of the facial canal in the internal auditory canal. In some cases, the arachnoid extended as far as the geniculate ganglion, and in rare cases, beyond it. The arachnoid around the eighth nerve ends at the lamina cribrosa. The location of psammoma bodies in the labyrinthine portion and the geniculate ganglion portion of the facial canal also corresponds to the areas supplied by the labyrinthine artery in the facial canal.[22] We suggest that psammoma bodies in the internal auditory canal may be formed by arachnoid cells. This interpretation is consistent with the finding that the intracranial subarachnoidal space for cerebrospinal fluid is present at least up to the geniculate ganglion portion and may be present even in more peripheral portions, such as the tympanic and mastoid portions of the facial canal. Also, it may be present around the SVN and IVN or CN up to the fundus of the internal auditory canal. This extension of the arachnoid space is a potential communication route for infection from the middle ear, through the dehiscence of the facial canal,[23] and through the facial canal and the internal auditory canal to both the inner ear and the intracranial subarachnoidal space.

The positive correlation between the number of psammoma bodies and age suggests that psammoma body formation may be a normal consequence of aging. This interpretation is consistent with previous reports that psammoma bodies are found in normal meninges, choroid plexus, and pineal gland of the aged.[2,4,12-16] The anatomic distribution of psammoma bodies in the internal auditory, facial, and singular canals raises the issue of their potential role in age-related dysfunction of these nerves. For example, findings of a psammoma body compressing the surface of the FN in the labyrinthine potion of the facial canal (Fig 9) and numerous psammoma bodies surrounding the posterior ampullary nerve of the IVN in the singular canal (Fig 10) suggest that psammoma bodies may be one of the factors associated with age-related symptoms of the seventh and eighth cranial nerves. Investigation in cases of documented seventh and eighth nerve dysfunction should be performed, since our results were obtained from asymptomatic cases.

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REFERENCES

1. Boldrey E. Pathology of the nervous system. Vol 2. New York, NY: McGraw-Hill, 1971:2125-44.

2. Kepes JJ. Meningiomas, biology, pathology and differential diagnosis. New York, NY: Masson, 1982:61-76.

3. Russell DS. Pathology of tumors of the nervous system. Baltimore, Md: Williams & Wilkins, 1989:449-529.

4. Klinck GH, Winship T. Psammoma bodies and thyroid cancer. Cancer 1959;12:656-62.

5. Batsakis JG, Nishiyama RH, Rich CR. Microlithiasis (calcospherites) and carcinoma of the thyroid gland. AMA Arch Pathol 1960;69:493-8.

6. Aure JC, Höeg K, Kolstad P. Psammoma bodies in serous carcinoma of the ovary: a prognostic study. Am J Obstet Gynecol 1971:109:113-8.

7. Patchefsky AS, Hoch WS. Psammoma bodies in diffuse toxic goiter. Am J Clin Pathol 1972;57:551-6.

8. Kepes JJ. Cellular whorls in brain tumors other than meningiomas. Cancer 1976;37:2232-7.

9. Unterman DH, Reingold IM. The occurrence of psammoma bodies in papillary adenocarcinoma of the lung. Am J Clin Pathol 1972;57:297-302.

10. Hameed K, Morgan DA. Papillary adenocarcinoma of

endometrium with psammoma bodies: histology and fine structure. Cancer 1972;29:1326-35.

11. Kuchemann K. Papillary cystadenoma of the epididymis with psammoma bodies. Beitr Pathol 1974;153:406-9.

12. Weitzner S. Intradermal nevus with psammoma body formation. Arch Dermatol 1968;98:287-9.

13. Virtanen I, Lehtonen E, Wartiovaara J. Structure of psammoma bodies of a meningioma in scanning electron microscopy. Cancer 1976;38:824-9.

14. Kubota T, Hirano A, Yamamoto S, Kajikawa K. The fine structures of psammoma bodies in meningocytic whorls. J Neuropathol Exp Neurol 1984;43:37-44.

15. Yamashima T, Kida T, Kubota T, Yamamoto S. The origin of psammoma bodies in the human arachnoid villi. Acta Neuropathol (Berl) 1986;71:19-25.

16. Alcolado JC, Moore IE, Weller RO. Calcification in the human choroid plexus, meningiomas and pineal gland. Neuropathol Appl Neurobiol 1986;12:235-50.

17. Sando I, Doyle WJ, Okuno H, Takahara T, Kitajiri M, Coury WJ III. A method for the histopathological analysis of the temporal bone and the eustachian tube and its accessory structures. Ann Otol Rhinol Laryngol 1986;95:267-74.

18. Rutka JT, Giblin J, Dougherty DV, McCulloch JR, DeArmond SJ, Rosenblum ML. An ultrastructural and immunocytochemical analysis of leptomeningeal and meningioma cultures. J Neuropathol Exp Neurol 1986;45:285-303.

19. Rabinovitch AL, Anderson HC. Biogenesis of matrix vesicles in cartilage growth plates. Fed Proc 1976;35:112-6.

20. Lipper S, Dalzell JC, Watkins PJ. Ultrastructure of psammoma bodies of meningioma in tissue culture. Arch Pathol Lab Med 1979;103:670-5.

21. Perlman HB, Lindsay JR. Relation of the internal ear spaces to the meninges. Arch Otolaryngol Head Neck Surg 1939;29:12-23.

22. Ogawa A, Sando I. Spatial occupancy of vessels and facial nerve in the facial canal. Ann Otol Rhinol Laryngol 1982;91:14-9.

23. Takahashi H, Sando I. Facial canal dehiscence: histologic study and computer reconstruction. Ann Otol Rhinol Laryngol 1992;101:925-30.

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XVI IBERO-AMERICAN CONGRESS OF NEUROLOGY

The XVI Ibero-American Congress of Neurology will be held in Rosario, Argentina, April 16-19, 2000. For information, contact Soledad Piuma, Congresos Internacionales, Moreno 584, 9P, Buenos Aires, Argentina; fax 54 11 3310223; e-mail spiuma@congresosint.com.ar.

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EXPRESSION OF NPY Y1 AND CGRP1 RECEPTORS IN HUMAN NASAL MUCOSA: IMPLICATIONS IN ALLERGIC RHINITIS

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Numerous nerve fibers containing neuropeptide Y (NPY) and calcitonin gene–related peptide (CGRP) have been found in the human nasal mucosa by means of immunocytochemistry. We detected NPY Y1 and CGRP1 receptors at the same location using reverse transcriptase–polymerase chain reaction. The possibility of local release in connection with specific target receptors suggests a role for endogenous NPY and CGRP in the regulation of vascular tone, glandular secretion, and epithelial functions.

KEY WORDS — calcitonin gene–related peptide, human, immunocytochemistry, neuropeptide Y, nose, polymerase chain reaction.

INTRODUCTION

It is well established that nasal obstruction and rhinorrhea are influenced by neuropeptides released from sympathetic, parasympathetic, and sensory nerves.[1] In the human nasal mucosa there is a rich supply of peptide-containing nerve fibers, of which those containing neuropeptide Y (NPY) and calcitonin gene–related peptide (CGRP) are prominent. The NPY-containing nerve fibers occur mainly around small blood vessels, arteries in particular,[2] whereas CGRP-containing fibers can be seen around blood vessels and nasal glands, as well as beneath and within the epithelium.[2,3] Neuropeptide Y is a potent vasoconstrictor,[4] and intranasal application of NPY reduces nasal obstruction and mucous secretion evoked by allergen challenge in allergic patients.[5,6] Calcitonin gene–related peptide takes part in sensory information.[7] Furthermore, in the human nose, CGRP increases nasal airway resistance, but seems to have no or only minor effects on glandular secretion.[8,9] At least 5 human NPY subreceptors have been cloned,[10] and 2 types of CGRP subreceptors have been postulated, of which 1 has been cloned and the other pharmacologically characterized.[11] A role for exogenous application of NPY or of NPY agonists in the treatment of allergic rhinitis has been suggested and to some extent evaluated, whereas therapeutic application of CGRP antagonists remains to be explored.[5]

In the present study, we have investigated the distribution of NPY- and CGRP-containing nerve fibers in the human nasal mucosa and evaluated the presence of messenger RNA (mRNA) for the 2 target receptors, NPY Y1 and CGRP1, in the area.

MATERIAL AND METHODS

Tissues. For immunocytochemistry, specimens from the human nasal mucosa (inferior nasal turbinate) were obtained in conjunction with nasal surgery (conchotomy) from 5 patients (age range 24 to 35 years). For RNA experiments, tissue was collected from another 5 patients (age range 24 to 50 years), frozen in liquid nitrogen immediately after acquisition, and stored at −70°C until use. The project was approved by the Ethics Committee of Lund University.

Immunocytochemistry. For the immunocytochemical demonstration of NPY and CGRP, indirect immunofluorescence was used. The NPY and CGRP antigens (Eurodiagnostica, Malmö, Sweden) were raised in rabbits and used at dilutions of 1:320 and 1:1,280, respectively. The sections were exposed to the primary antiserum overnight at 4°C in a moist chamber. The site of the antigen-antibody reaction was revealed by application of fluorescein isothiocyanate–labeled antibodies raised against immunoglobulin G (Dako, Copenhagen, Denmark) at a dilution of 1:320 for 1 hour at room temperature. Control sections were exposed to antiserum that had been preadsorbed with an excess amount of the antigen (10 to 100 µg of NPY or CGRP per milliliter of diluted antiserum). Additionally, the antiserum was tested for cross-reaction with other peptides (10 to 100 µg of peptide per milliliter diluted antiserum). No such cross-reaction was found. Cross-reactions

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with still other peptides or proteins containing amino acid sequences recognized by the different antisera cannot be excluded. It is appropriate, therefore, to refer to the immunoreactive material as NPY-like or CGRP-like. For brevity, the shorter terms NPY and CGRP are used.

Isolation of Total RNA. Total cellular RNA was extracted with TRIzol reagent (Gibco BRL, Life Technologies, Sweden). Frozen tissue was homogenized for 30 to 60 seconds with 1 mL of TRIzol reagent at room temperature until completely disrupted, by means of the microprobe of a power homogenizer (Polytron Kinematica AG, model PT 1200, Labora, Sweden). The homogenates were mixed with chloroform and centrifuged at 12,000*g* for 15 minutes at 4°C. The aqueous phase, containing RNA, was transferred to a fresh tube, and the RNA was precipitated from the aqueous phase by the addition of isopropanol. Samples were incubated at room temperature for 10 minutes and centrifuged at 12,000*g* for 10 minutes at 4°C.

The RNA pellet was finally washed with 70% ice-cold ethanol, air-dried, dissolved in 20 µL of diethylpyrocarbonate-treated water, and stored at −20°C until use. The purity and yield of total RNA were determined spectrophotometrically by measurement of optical density of an aliquot at 260 nm and 280 nm, by means of a DU-65 spectrophotometer (Beckman Instruments, Sweden). The ratio of absorption (260: 280) of all preparations was between 1.6 and 1.8. Finally, samples were subjected to gel electrophoresis and stained with ethidium bromide to prove the integrity of the 18 and 28 S ribosomal RNAs.

Removal of Genomic DNA From RNA Samples. In order to eliminate eventual residual contaminating DNA that might produce a false-positive amplification signal, duplicate tubes containing 1 µg of total RNA were pretreated with 1 U of amplification-grade DNase I (Gibco BRL, Life Technologies, Sweden) in DNase I reaction buffer, in the presence of 20 U of RNase inhibitor (Perkin-Elmer AB, Sweden). RNase-free water was added to each tube to a final volume of 10 µL, and samples were incubated for 15 minutes at room temperature. One microliter of 25-mmol/L ethylenediaminetetraacetic acid (EDTA) solution (pH 8.0) was added to each tube, and samples were heated for 10 minutes at 65°C to inactivate the DNase I and thereafter immediately cooled on ice.

Reverse Transcriptase–Polymerase Chain Reaction. Synthesis of first-strand complementary DNA (cDNA) and subsequent polymerase chain reaction (PCR) amplification were carried out with the GeneAmp RNA PCR kit reagents (Perkin-Elmer AB, Sweden) in a PCR DNA thermal cycler (Perkin-Elmer AB, Sweden).

DNase-treated RNA samples were reverse-transcribed to cDNA in a 20-µL reaction volume in the presence of PCR buffer (50 mmol/L potassium chloride, 10 mmol/L Tris–hydrochloric acid, pH 8.3), 5 mmol/L magnesium chloride, 1 mmol/L of deoxyribonucleoside triphosphate (dNTP), 50 pmol of oligo (dT) primers, and 50 U of M-MLV (Moloney murine leukemia virus) reverse transcriptase. The reaction was incubated at 42°C for 15 minutes, heated to 99°C for 5 minutes, and chilled to 5°C for 5 minutes.

The PCR reaction was performed with specific oligonucleotide primers and according to published sequences of the human NPY Y1 and CGRP1 receptors, and designed by computer analysis on Oligo primer analysis software, version 4.0. The primers used to amplify a 350 base pair (bp) fragment of the human NPY Y1 receptor mRNA sequences corresponding to a region in the vicinity of TM5 and TM7 and spanning the second intron located exactly after TM5 were as follows: sense primer, 5'-TAT ACC ACT CTC CTC TTG GTG CTG-3'; and antisense primer, 5'-CTG GAA GTT TTT GTT CAG GAA CCC A-3'. The primers used to amplify a fragment of the human CGRP1 receptor generating a 339 bp product were as follows: sense primer, 5'-TCT GGT TCT CTT GCC TTT TTT TAT G-3', corresponding to nucleotides 581 to 605; and antisense primer, 5'-GTC CAT GTT CTG TTG CTT GCT G-3', corresponding to nucleotides 898 to 919.

The PCR amplification reaction for NPY Y1 was composed of 2 µL of the first-strand cDNA reaction mixture and 48 µL master mix containing PCR buffer, 1.5 mmol/L magnesium chloride, 1.0 µmol/L of each sense and antisense specific primer, and 2.0 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer AB, Sweden). The PCR amplification reaction for CGRP1 was composed of 2 µL of the first-strand cDNA reaction mixture and 48 µL master mix containing PCR buffer, 1.0 mmol/L magnesium chloride, 0.2 µmol/L of each sense and antisense primer, and 1.2 U of AmpliTaq Gold DNA polymerase. The PCR for NPY Y1 was performed under the following conditions: 9 minutes at 95°C before undergoing 35 cycles of 1 minute at 94°C and 1 minute at 60°C, followed by a 7-minute final extension at 72°C. The PCR used for CGRP1 was 9 minutes at 94°C before undergoing 35 cycles of 1 minute at 94°C and 1 minute at 63°C, followed by a 7-minute final extension at 72°C.

Electrophoretic Analysis. Ten microliters from all PCR-amplified products were electrophoretically separated on a 1.5% agarose gel (Gibco BRL, Life Technologies, Sweden) containing 0.5 µg/mL ethid-

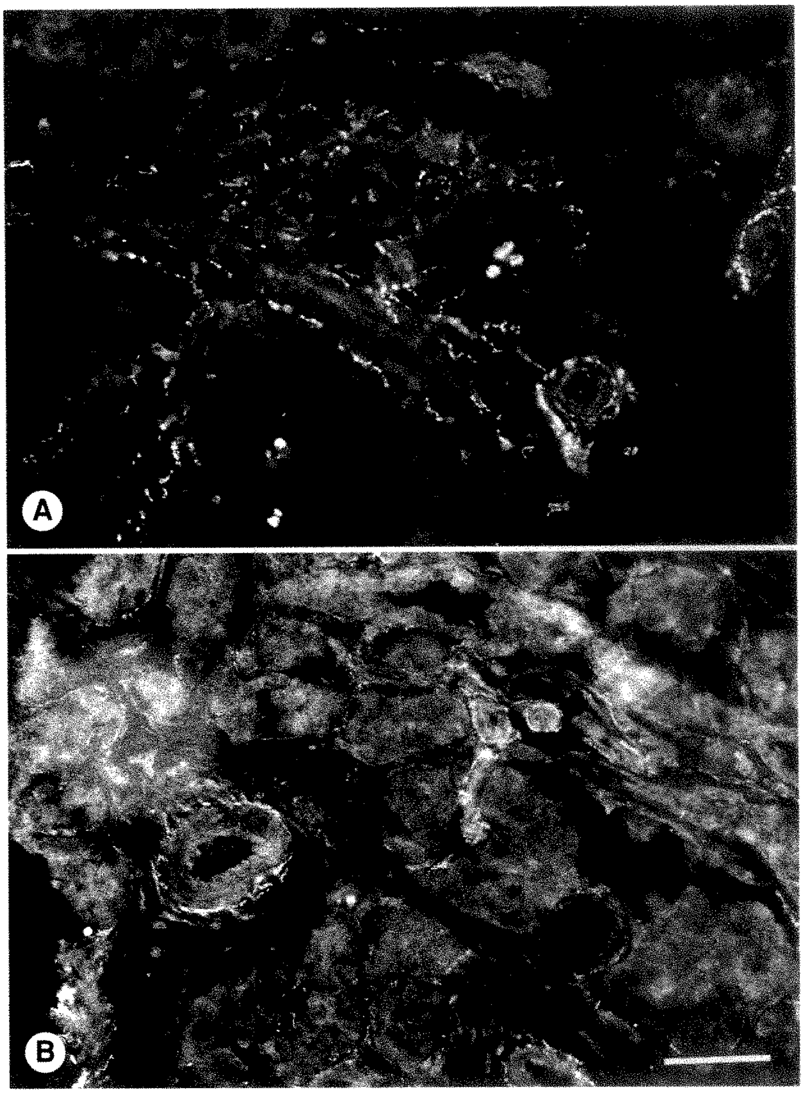

Fig 1. Human nasal mucosa. Size bar — 50 µm. A) Fine-beaded neuropeptide Y (NPY)–containing nerve fibers are seen around small blood vessels and in stroma. B) Moderate supply of calcitonin gene–related peptide–containing nerve fibers around small blood vessel.

ium bromide (Sigma E 1510) in TBE buffer (89 mmol/L TRIS-borate, 2 mmol/L EDTA, pH 8.0) at 5 V/cm for 1 1/2 hours. This analysis was performed in a 20 × 10-cm Midicell, model EC 350 (E-C Apparatus Corporation; Techtum Lab AB, Sweden). A 100 bp DNA ladder (Promega, SDS, Sweden) was run in each of the outside lanes to confirm the molecular size of the amplification product.

RESULTS

Numerous fine-beaded, NPY-containing nerve fibers were seen in the nasal mucosa. The majority of the immunoreactive nerve fibers were found in connection with blood vessels (Fig 1). Occasional fibers were also seen in the stroma without apparent association to blood vessels. We saw CGRP-containing fibers close to small blood vessels and seromucous glands within and beneath the epithelium (Fig 1).

Agarose gel electrophoresis of the reverse transcriptase (RT)–PCR products from the human nasal mucosa demonstrated products of the expected sizes, corresponding to mRNA encoding the human CGRP1 receptor (339 bp) lane 5 and the human NPY Y1 receptor (350 bp) lane 3 (Fig 2). To determine if the amplification product came exclusively from the RNA, an RT-negative reaction was run in which the enzyme was replaced by RNase-free water in lanes 2 and 4.

DISCUSSION

Neuropeptide Y is a neuropeptide widely distributed in the central and peripheral nervous systems. It modulates several physiological processes, including appetite, blood pressure, and circadian rhythms.[12] There is a rich supply of NPY-immunoreactive nerve fibers around peripheral blood vessels.[13] In sympathetic nerve cell bodies and around blood vessels, NPY coexists with tyrosine hydroxylase and dopamine-β-hydroxylase, markers for adrenergic neurons.[14] The NPY-mediated vasoconstriction is long-lasting and resistant to α-adrenoceptor antagonists. In peripheral blood vessels, NPY may act on vessel

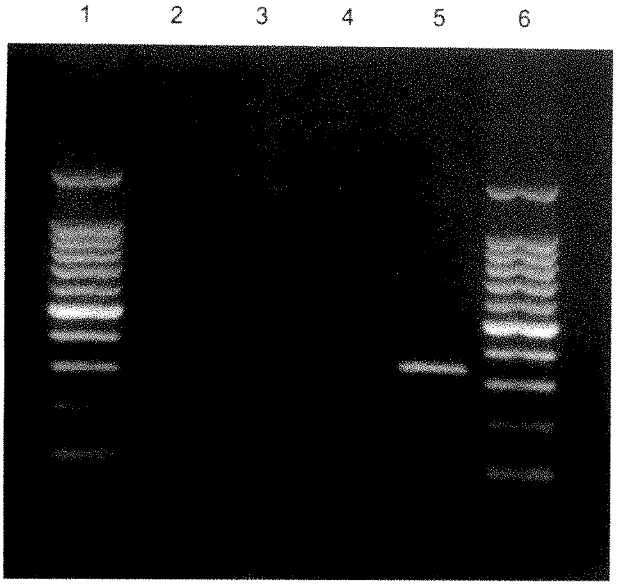

Fig 2. Gel electrophoresis of reverse transcriptase–polymerase chain reaction products after 35 cycles of amplification of messenger RNA fragments corresponding to human NPY Y_1 receptor transcripts. As positive control, human uterine placenta was used (lanes 3 and 4). As negative control, no amplification product occurred when reverse transcriptase was omitted in first-strand complementary DNA reaction. Promega's 100 base pair DNA ladder was run to confirm molecular size of amplification product (lane 1).

tone by direct constriction,[15] by potentiation of noradrenaline-induced contraction,[16] or by inhibition of presynaptic noradrenaline release.[17,18] It has recently been demonstrated that activation of the NPY Y_1 receptor results in contraction of human cerebral and subcutaneous arteries[19,20] and potentiation of noradrenaline-induced contraction in human omental blood vessels.[21] Thus, it is likely that this receptor is involved in the NPY responses in the nasal mucosa. Neuropeptide Y exerts its action through several classes of subreceptors.[10] The roles played by each of the different subreceptors have been difficult to delineate due to the lack of selective receptor antagonists. However, by using differences in the sensitivity to various NPY analogs, two NPY subreceptors, Y_1 and Y_2, have been functionally characterized. The mRNA for the Y_1 receptor has been demonstrated in human arteries.[19,20,22] In the human nose, protective effects of local NPY application on capsaicin-induced sneezing and secretion have been described and may partly be related to both Y_1 receptor–mediated contraction and NPY-mediated inhibition of neurotransmitter release.[5,23] In the present study, numerous NPY-immunoreactive nerve fibers were seen around blood vessels, corresponding to previous findings in the nasal mucosa of laboratory animals and humans.[2,24]

Nerve fibers containing CGRP were seen around blood vessels and nasal glands and within and beneath the epithelium in the human nasal mucosa, confirming previous results.[2,3] In laboratory animals, CGRP and NPY are up-regulated in response to peripheral inflammation[25]; this finding suggests that both peptides are involved in the response to inflammation and nociception. In addition, in laboratory animals, CGRP may dilate blood vessels and regulate glandular secretion.[26,27] Highly specific CGRP receptors have been identified both in the intima and in the media layers of peripheral blood vessels, such as the superior mesenteric, the femoral, and the coronary arteries.[28,29] Calcitonin gene–related peptide causes marked stimulation of adenylate cyclase in cultured aortic smooth muscle and endothelial cells. These binding sites are linked to the generation of cyclic AMP via a non–endothelium-dependent mechanism.[30] The C-terminal fragment of human α-CGRP, hCGRP$_{8-37}$, has antagonistic effects on CGRP-induced adenylate cyclase activity in liver cell membranes and acts as a competitive α-hCGRP receptor antagonist in the cardiovascular system of the rat, in the guinea pig atrium, and in the guinea pig ileum.[31,32] hCGRP$_{8-37}$ shows a weak antagonistic effect in rat vas deferens and is ineffective against α-hCGRP–induced hyperthermia. Consequently, the existence of at least two classes of CGRP receptors has been suggested,[31] one being sensitive and the other being insensitive to hCGRP$_{8-37}$. The CGRP$_1$ receptor is highly sensitive to the antagonistic properties of CGRP$_{8-37}$. Receptors that do not respond to CGRP$_{8-37}$ are at present grouped together as CGRP$_2$.[32,33] Thus, our finding of CGRP$_1$ receptor mRNA in the nasal mucosa agrees well with previous pharmacological studies that have shown a potent relaxant CGRP$_1$ receptor coupled to adenylyl cyclase activity.[34]

The presence of NPY-containing nerve fibers around blood vessels in the human nasal mucosa, together with our detection of NPY Y_1 receptor mRNA in the same area and the known effects of NPY, makes it conceivable that NPY Y_1 receptors play a role in the regulation of nasal airway resistance and nasal secretion. Neuropeptide Y agonists could therefore be of therapeutic interest in the treatment of allergic rhinitis. The finding of CGRP-containing fibers close to glands, around blood vessels, and beneath and within the epithelium and the known vasodilatory effects of CGRP make it conceivable that part of the vasodilatation is mediated by a CGRP$_1$ receptor. Since CGRP gives rise to increased nasal mucosal blood flow, tentatively via the CGRP$_1$ receptor, it is tempting to speculate that a CGRP$_1$ receptor antagonist might have a beneficial effect on the symptoms of allergic rhinitis.

REFERENCES

1. Barnes P, Baraniuk JN, Belvisi MG. Neuropeptides in the respiratory tract. Am Rev Respir Dis 1991;144:1187-98.
2. Hauser-Kronberger C, Hacker GW, Muss W, Saria A, Albegger K. Autonomic and peptidergic innervation of human nasal mucosa. Acta Otolaryngol (Stockh) 1993;113:387-93.
3. Baraniuk JN, Lundgren JD, Goff J, et al. Calcitonin gene–related peptide in human nasal mucosa. Am J Physiol 1990;258: L81-L88.
4. Edvinsson L, Håkanson R, Wahlestedt C, Uddman R. Effects of neuropeptide Y on the cardiovascular system. Trends Pharmacol Sci 1987;8:231-5.
5. Baraniuk JN, Silver PB, Kaliner MA, Barnes P. Neuropeptide Y is a vasoconstrictor in human nasal mucosa. J Appl Physiol 1992;73:1867-72.
6. Lacroix JS, Mosimann B. Attenuation of allergen-evoked nasal responses by local pretreatment with exogenous neuropeptide Y in atopic patients. J Allergy Clin Immunol 1996;98:611-66.
7. Lundberg JM, Franco-Cereceda A, Hua X-Y, Hökfelt T, Fischer J. Co-existence of substance P and calcitonin gene–related peptide immunoreactivities in sensory nerves in relation to cardio-vascular and broncho-constrictor effects of capsaicin. Eur J Pharmacol 1985;108:315-9.
8. Guarnaccia S, Baraniuk JN, Bellanti J, Duina M. Calcitonin gene–related peptide nasal provocation in humans. Ann Allergy 1994;72:515-9.
9. Chatelain C, Pochon N, Lacroix JS. Functional effects of phosphoramidon and captopril on exogenous neuropeptides in human nasal mucosa. Eur Arch Otorhinolaryngol 1995;252: 83-5.
10. Blomqvist AG, Herzog H. Y-receptor subtypes — how many more? Trends Neurosci 1997;20:294-8.
11. Aiyar N, Rand K, Elshourbagy NA, et al. A cDNA encoding the calcitonin gene–related peptide type 1 receptor. J Biol Chem 1996;271:11325-9.
12. Wahlestedt C, Reis DJ. Neuropeptide Y–related peptides and their receptors — are the receptors potential therapeutic drug targets? Annu Rev Pharmacol Toxicol 1993;32:309-52.
13. Uddman R, Ekblad E, Edvinsson L, Håkanson R, Sundler F. Neuropeptide Y–like immunoreactivity in perivascular nerve fibres of the guinea-pig. Regul Pept 1985;10:243-57.
14. Lundberg JM, Terenius L, Hökfelt T, Goldstein M. High levels of neuropeptide Y in peripheral noradrenergic neurons in various mammals including man. Neurosci Lett 1983;42:167-72.
15. Lundberg JM, Tatemoto K. Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to sympathetic vasoconstriction resistant to α-adrenoceptor blockade. Acta Physiol Scand 1982;116:393-402.
16. Edvinsson L, Ekblad E, Håkanson R, Wahlestedt C. Neuropeptide Y potentiates the effect of various vasoconstrictor agents on rabbit blood vessels. Br J Pharmacol 1984;83:519-25.
17. Lundberg JM, Stjärne L. Neuropeptide Y (NPY) depresses the secretion of 3H-noradrenaline and the contractile response evoked by field stimulation in the rat vas deferens. Acta Physiol Scand 1984;120:477-9.
18. Linton-Dahlöf P. Modulatory interactions of neuropeptide Y (NPY) on sympathetic neurotransmission. Acta Physiol Scand Suppl 1989;137(suppl 586).
19. Nilsson T, Cantera L, Edvinsson L. Presence of neuropeptide Y Y_1 receptor mediating vasoconstriction in human cerebral arteries. Neurosci Lett 1996;204:145-8.
20. Nilsson T, Erlinge D, Cantera L, Edvinsson L. Contractile effects of neuropeptide Y in human subcutaneous resistance arteries are mediated by Y_1 receptors. J Cardiovasc Pharmacol 1996;28:764-8.
21. Bergdahl A, Nilsson T, Cantera L, et al. Neuropeptide Y potentiates noradrenaline-induced contraction through the neuropeptide Y Y_1 receptor. Eur J Pharmacol 1996;316:59-64.
22. Larhammar D, Blomqvist AG, Yee F, Jazin E, Yoo H, Wahlestedt C. Cloning and functional expression of a human neuropeptide Y/peptide YY receptor of the Y_1 type. J Biol Chem 1992;267:10935-8.
23. Lacroix JS, Ricchetti AP, Morel D, Mossimann B, Waeber B, Grouzmann E. Intranasal administration of neuropeptide Y in man: systemic absorption and functional effects. Br J Pharmacol 1996;118:2079-84.
24. Uddman R, Sundler F, Emson P. Occurrence and distribution of neuropeptide Y–immunoreactive nerves in the respiratory tract and middle ear. Cell Tissue Res 1984;237:321-7.
25. Ji R-R, Zhang X, Wiesenfeld-Hallin Z, Hökfelt T. Expression of neuropeptide Y and neuropeptide Y (Y_1) receptor mRNA in rat spinal cord and dorsal root ganglia following peripheral tissue inflammation. J Neurosci 1994;14:6423-34.
26. Stjärne P, Lundblad L, Änggård A, Hökfelt T, Lundberg T. Tachykinins and calcitonin gene–related peptide: co-existence in sensory nerves of the nasal mucosa and effects on blood flow. Cell Tissue Res 1989;256:439-46.
27. Gawin AZ, Kaliner MA, Baraniuk JN. Effects of substance P and calcitonin gene–related peptide on guinea pig nasal mucosa secretion in vivo. Acta Otolaryngol (Stockh) 1993; 113:533-9.
28. Sigrist S, Franco-Cereceda A, Muff R, Henke H, Lundberg JM, Fisher JA. Specific receptor and cardiovascular effects of calcitonin gene–related peptide. Endocrinology 1986; 119:381-7.
29. Wimalawansa SJ, Emson PC, MacIntyre I. Regional distribution of calcitonin gene–related peptide and its specific binding sites in rats with particular reference to the nervous system. Neuroendocrinology 1987;46:131-6.
30. Edvinsson L, Fredholm BB, Hamel E, Jansen I, Verrecchia C. Perivascular peptides relax cerebral arteries concomitant with stimulation of cyclic adenosine monophosphate accumulation or release of an endothelium-derived relaxing factor in the cat. Neurosci Lett 1985;58:213-7.
31. Dennis T, Fournier A, Cadieux A, et al. hCGRP$_{8-37}$, a calcitonin gene–related peptide antagonist revealing calcitonin gene–related peptide receptor heterogeneity in brain and periphery. J Pharmacol Exp Ther 1990;254:123-8.
32. Donoso VM, Fournier A, St Pierre S, Huidobro-Toro JP. Pharmacological characterization of CGRP$_1$ receptor subtype in the vascular system in the rat: studies with hCGRP fragments and analogs. Peptides 1990;11:885-9.
33. Jansen I. Characterization of calcitonin-gene–related peptide (CGRP) receptors in guinea-pig basilar arteries. Neuropeptides 1991;21:73-9.
34. Jansen-Olesen I, Mortensen A, Edvinsson L. Calcitonin gene–related peptide is released from capsaicin-sensitive nerve fibres and induces vasodilatation of human cerebral arteries concomitant with activation of adenylyl cyclase. Cephalalgia 1996; 16:310-6.

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MINERALOCORTICOID RECEPTORS IN THE MAMMALIAN OLFACTORY MUCOSA

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Mineralocorticoid hormones regulate secretion and absorption in a wide variety of epithelial tissues, although specific mechanisms in the olfactory mucosa are currently unknown. Utilizing reverse transcription–polymerase chain reaction (RT-PCR) analysis, we have demonstrated the expression of mineralocorticoid (type I) receptor messenger RNA in the rodent olfactory mucosa. Amplification products of predicted size were obtained with nucleotide sequences corresponding to respective mineralocorticoid receptor (MR) kidney transcripts. Immunocytochemistry, using an antibody with known specificity for MRs, was then utilized in order to localize the cellular site(s) of MR protein expression in the olfactory mucosa. The highest levels of MR immunoreactivity were localized to the supranuclear region of sustentacular cells, as well as the acinar cells of the Bowman's glands. The respiratory regions of the nasal cavity were devoid of appreciable MR immunoreactivity. This study demonstrates both MR transcript and protein expression in the olfactory mucosa. We hypothesize that the mineralocorticoid hormones may have a role in modulation of olfactory secretion and/or sensory transduction in the peripheral olfactory system.

KEY WORDS — aldosterone, corticosteroids, Na,K-ATPase, neurons, olfactory mucus, olfactory receptor cells, sustentacular cells.

INTRODUCTION

The olfactory mucosa is a neuroepithelial tissue wherein the secretory and absorptive functions subserve sensory transduction and transmission. In mammals, acinar cells of Bowman's glands are the primary source of olfactory mucus, which is a highly specialized substance vastly different from nasal respiratory secretions.[1] Olfactory mucus bathes the receptor cilia of olfactory neurons and provides the source of ions necessary for transduction. Sustentacular cells mediate the flux of water and electrolytes across the epithelial surface and also serve glial-like functions, regulating the ion content of perineural fluid surrounding the olfactory neurons.[2] Despite the proximity of the olfactory mucosa to the external environment, these specialized secretory processes evidently maintain the necessary conditions for the perception of odor.

Olfactory secretion and absorption results in the generation of 2 distinct microenvironments: perineural fluid, which covers the lateral plasma membranes of the receptor cells, and olfactory mucus, which covers the dendritic knobs and cilia of the receptive field. These 2 compartments are separated by tight junctions between sustentacular cells and neurons at the apical surface.[3] The characteristics of the extracellular (perineural) fluid and olfactory mucus surrounding receptor cells are important for olfactory transduction and transmission.[1,2] Studies focused on the regulation of olfactory secretion have stressed the action of specific transmitters and modulatory substances acting through an extensive neural input to Bowman's glands.[2,4] The possibility of systemic (hormonal) regulation of these processes has not been evaluated.

Mineralocorticoids are 1 of 2 broad classes of steroid hormones produced by the adrenal cortex that have been shown to regulate secretion in a variety of epithelial tissues.[5,6] These hormones act by binding to specific receptors located in target cells. The major physiological mammalian mineralocorticoid is aldosterone, and recent biochemical studies have dem-

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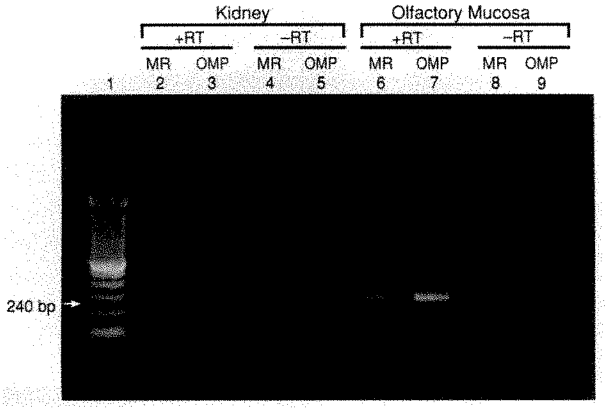

Fig 1. Reverse transcription–polymerase chain reaction analysis of mineralocorticoid receptor (MR) and olfactory marker protein (OMP) expression from total RNA of olfactory mucosa and kidney (positive control). This Figure shows ethidium bromide–stained 1.5% agarose gels containing complementary DNA (cDNA) amplified from reverse-transcribed RNA by means of PCR primers for MR and OMP as denoted above each lane. 100 kilobase DNA ladder (Gibco BRL, Gaithersburg, Md) was included at left of gel (lane 1). cDNA bands of predicted sizes were obtained for MR and OMP (lanes 2, 6, and 7), demonstrating adequately functioning primers. When RT step was omitted (negative control), no bands were observed (lanes 4, 5, 8, and 9). Lane 3 shows absence of OMP in kidney, as expected.

onstrated the presence of high levels of aldosterone binding within the olfactory mucosa,[7] whereas in other epithelial tissues, aldosterone acts, in part, by increasing the activity of the enzyme sodium- and potassium-activated adenosinetriphosphatase (Na,K-ATPase).[5,6] Recent animal studies have suggested that olfactory Na,K-ATPase activity is regulated by aldosterone as well.[8] To further evaluate the possible role of this hormone in the peripheral processes of olfaction, we utilized the polymerase chain reaction (PCR) to demonstrate the presence of messenger RNA (mRNA) for the mineralocorticoid receptor (MR) within the olfactory mucosa. Second, we investigated the distribution of the MR protein expression within the cells of the mucosa by utilizing an antibody specific for this receptor.

METHODS

Animal Model. Guinea pigs, mice, and rats were used as animal subjects in the current experiments, since each species has certain advantages and disadvantages in the investigation of corticosteroids and the olfactory system. Initial experiments on this topic utilized guinea pigs as the subject model.[7,9] Guinea pigs were chosen due to their known similarity to humans with regard to corticosteroid biochemistry[7,9,10] and in view of their thin nasal bones and relatively wide nasal cavity. These latter qualities facilitate dissection of olfactory tissues for subsequent histologic studies. Mice also have thin nasal bones, but their corticosteroid biochemistry is similar to that of the rat, with corticosterone hormone being the primary glucocorticoid, and not cortisol, as in guinea pigs and humans.[7,9,10] As a result, guinea pigs and mice were chosen for the immunocytochemical experiments in this investigation. The rat is the standard animal model for molecular biological experiments, and as a consequence, the nucleotide sequence for rat MR is already known. Specific PCR primers were designed based on those data.[11] Finally, observation in different species allows a comparison of the data between species and also enhances the confidence in the accuracy of our observations.

Reverse Transcription–Polymerase Chain Reaction (RT-PCR). To demonstrate the expression of MR mRNA in the olfactory mucosa, 1 male adult Wistar rat per preparation (Charles River Breeding Laboratories, Inc, Kalamazoo, Mich) was anesthetized, and the nasal mucosa was quickly removed and stored at −70°C. Total RNA was isolated (Qiagen, Chatsworth, Calif), treated with DNAse I (Life Technologies, Gaithersburg, Md) to remove any residual DNA contamination, and reverse-transcribed. Reverse transcription was carried out with random hexamer primers and Moloney murine leukemia virus reverse transcriptase (Clontech, Palo Alto, Calif). The forward primer was 5'-CCAAAGGCTACCACAGTCTC-3', and the reverse primer was 5'-TCCCAGACCGACTATTGTCT-3', corresponding to nucleotide positions 481 to 500 and 720 to 701, respectively. The primer pair was specifically designed to amplify a 240 base pair (bp) fragment of the hormone binding domain. Expression of the olfactory marker protein (OMP) mRNA was used as a positive molecular control to indicate the presence of olfactory-specific transcripts in the RNA isolated from peripheral olfactory tissues. Rat OMP primers were selected from the coding region of the cloned rat OMP complementary DNA (cDNA) sequence.[12] The forward primer was 5'-GTTCTGGACCAGGACCTGAC-3' (nucleotides 42 to 62), and the reverse primer was 5'-CATGAGGTTGGTGAGGTCTG-3' (nucleotides 263 to 282). For positive MR controls, total RNA from rat kidney was utilized. Eight separate olfactory mucosa and 8 kidney RNA preparations were employed for Fig 1, and the results were reproducible across the preparations. A PCR analysis was initiated with a single period of denaturation at 95°C for 2 minutes, followed by 30 cycles of 94°C for 60 seconds, 55°C for 30 seconds (annealing), and 72°C for 60 seconds. This procedure was completed by continuation of the last 72°C incubation for a further 5 minutes.

Immunocytochemistry. Tissues from post-weaned mice (The Jackson Laboratory, Bar Harbor, Me), 15 to 18 days old, and male Hartley guinea pigs, 200 to

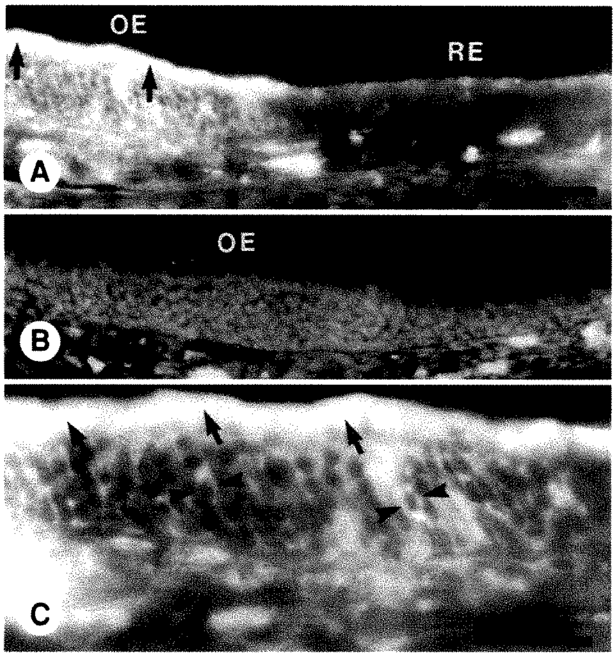

Fig 2. Light microscopic localization of MR-like immunoreactivity in mouse olfactory epithelium. A) Within mouse nasal cavity, fluorescein-labeled MR immunoreactivity is seen associated with apical region (arrows) of olfactory epithelium (OE). In contrast, MR-like immunoreactivity is absent from respiratory epithelium (RE). Bar (also for B) — 100 µm. B) When primary antibody was omitted from incubation medium, no fluorescent signal could be detected. C) Apical staining pattern identified in OE of A is associated with supranuclear region (arrows) of sustentacular cells. In addition, staining is seen in perinuclear region of probable olfactory receptor cells (large arrowheads) and axonal processes (small arrowheads) as well. Bar — 40 µm.

250 g (Charles River Breeding Laboratories, Inc), were fixed by vascular perfusion with 4% paraformaldehyde in 0.1-mol/L phosphate-buffered saline (PBS), pH 7.4 (for immunofluorescent studies), or with 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1-mol/L PBS, pH 7.4 (for immunoperoxidase studies), at room temperature. Either the nasal chambers were removed in situ and later decalcified, or the olfactory mucosa (directly anterior to the olfactory bulb on the roof of the nasal cavity) was dissected away from the bony tissues. The tissues were fixed by immersion for 1 additional hour at 4°C. Following fixation, tissues were rinsed in PBS, pH 7.4. The dissected olfactory mucosae were immediately processed for cryostat sectioning, while the whole nasal chambers were decalcified in a 3% ethylenediaminetetraacetic acid solution over 5 to 7 days at 4°C.[13] The tissues were then infiltrated with chilled phosphate-buffered graded (10% to 20% to 30%) sucrose solutions, embedded in OCT compound (Miles, Elkhart, Ind), and sectioned at 6 to 8 µm with a cryostat. Decalcified tissues were sectioned in the coronal plane.

The cDNA sequence of human MR has been determined,[14] and Krozowski et al[15] have synthesized an immunogenic segment of MR protein deduced from this clone. Polyclonal antibody, obtained by immunization of rabbits with a synthetic fusion protein corresponding to 167 amino acids of the N-terminal region of the human renal MR, has subsequently been used to localize MRs in ion-transporting epithelia (kidney, colon, and salivary and sweat glands) and nonepithelial tissue (neurons of the hippocampus) by means of immunohistochemical and Western blot analysis.[16,17] This same rabbit polyclonal antibody is used in this study.

After sectioning, tissues were rehydrated with 0.01-mol/L PBS for 15 minutes at room temperature. They were quenched for free aldehyde groups and endogenous peroxidase activity with 1% sodium borohydride in PBS and 0.3% hydrogen peroxide in absolute methanol, respectively. The sections were then incubated overnight in primary antibody at 4°C. The primary antibody (rabbit polyclonal antibody raised against MR protein) was diluted (1:200 to 1:1,000) with PBS containing 1% normal goat serum and 0.2% Triton X-100. For immunofluorescence detection, following incubation with the primary antibody, cryostat sections were incubated with a fluorescein-labeled goat anti-rabbit IgG (Zymed Immunochemical Co, Burlingame, Calif), rinsed in PBS, and mounted in a PBS solution containing 0.1% *p*-phenylenediamine and 70% glycerol. For immunoperoxidase detection, sections exposed to the primary antibody were incubated with a biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame). The biotin was then linked to peroxidase with a peroxidase-labeled ABC kit (Vector Laboratories). Peroxidase activity was localized with diaminobenzidine and hydrogen peroxide (Zymed Immunochemical Co, Los Angeles, Calif) used as substrates. The peroxidase reaction was terminated by 3 rinses in PBS, and slides were mounted in aqueous mounting medium (Sigma Chemical Co, St Louis, Mo). The slides were examined and photographed by light field or epifluorescence with a Leitz microscope. As a control, the primary anti-MR antibody was omitted from the incubation medium. The Animal Investigation Committee of Northwestern University approved the care and use of the animals employed in this study.

RESULTS

Expression of MR mRNA in Olfactory Mucosa. Synthesized PCR primers were effective in directing amplification of MR sequence from rat kidney cDNA and yielded bands of predicted molecular size, providing a positive control and demonstrating the functionality of the primers (Fig 1, lane 2). When the PCR

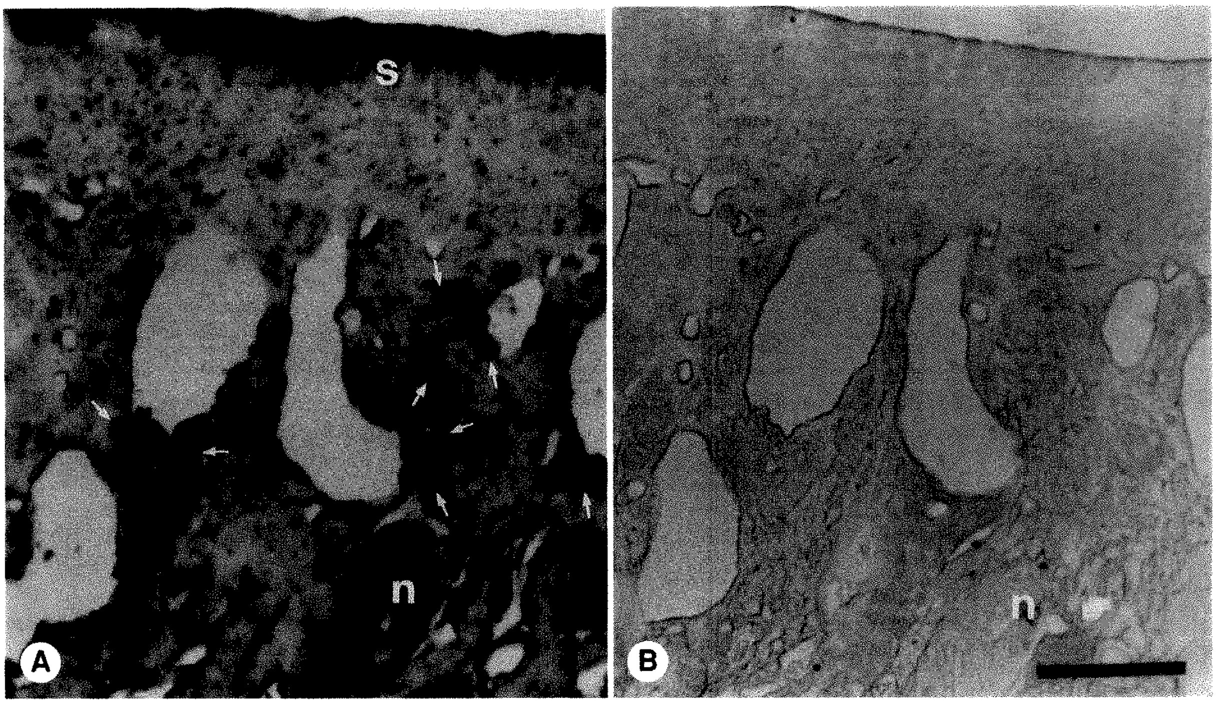

Fig 3. Light microscopic localization of MR-like immunoreactivity in guinea pig olfactory mucosa. Bar — 45 μm. A) This Figure is low-power micrograph showing olfactory epithelium and lamina propria of guinea pig. By use of immunoperoxidase technique, immunoreactivity for MRs is again clearly seen at apical surface (s) corresponding to sustentacular cells. Olfactory nerve axon bundles (n) and Bowman's glands (arrows) in lamina propria also stained for MRs. B) There is no staining in cells of epithelium or lamina propria when primary antibody is omitted from incubation medium.

reaction was performed with cDNA from rat olfactory mucosa mRNA, product corresponding to the MR was amplified (lane 6). The fact that the olfactory band represented actual MR message was confirmed by nucleotide sequence analysis. Product of the predicted 240 bp sequence for OMP (arrow) was amplified from the RNA extracted from the olfactory mucosa (lane 7), while no amplification above

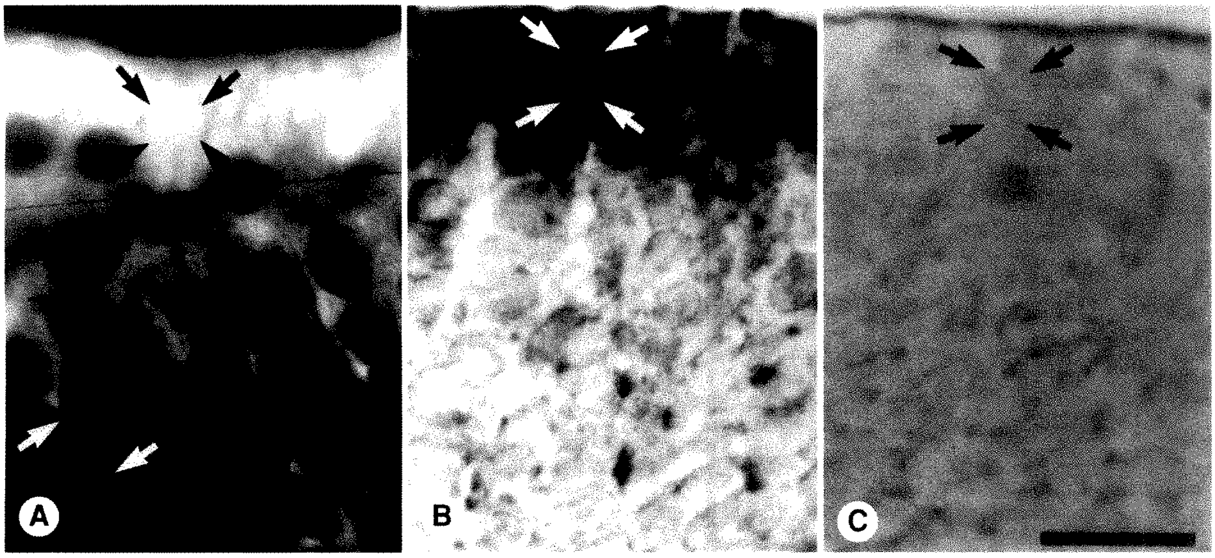

Fig 4. Light microscopic localization of MR-like immunoreactivity in rodent olfactory epithelium. Bar — 15 μm. A) This Figure shows higher-power micrograph of area indicated in Fig 2C. Upon closer examination, it is clear that supranuclear region (black arrows) of sustentacular cells and perinuclear region of olfactory receptor cells (arrowheads) and their axonal processes (white arrows) are positive for MR-like immunoreactivity. B) Similar localization of MR-like immunoreactivity was seen by immunoperoxidase (ABC) method in guinea pig. Immunocytochemical localization of MRs was clearly observed in supranuclear region (arrows) of sustentacular cells. Scattered precipitate is also seen in lower regions of epithelium, suggesting activity in olfactory receptor neurons. C) There is no staining of structures identified by immunoperoxidase technique in guinea pig olfactory mucosae (arrows) when polyclonal antibody to MR is omitted from primary incubation medium.

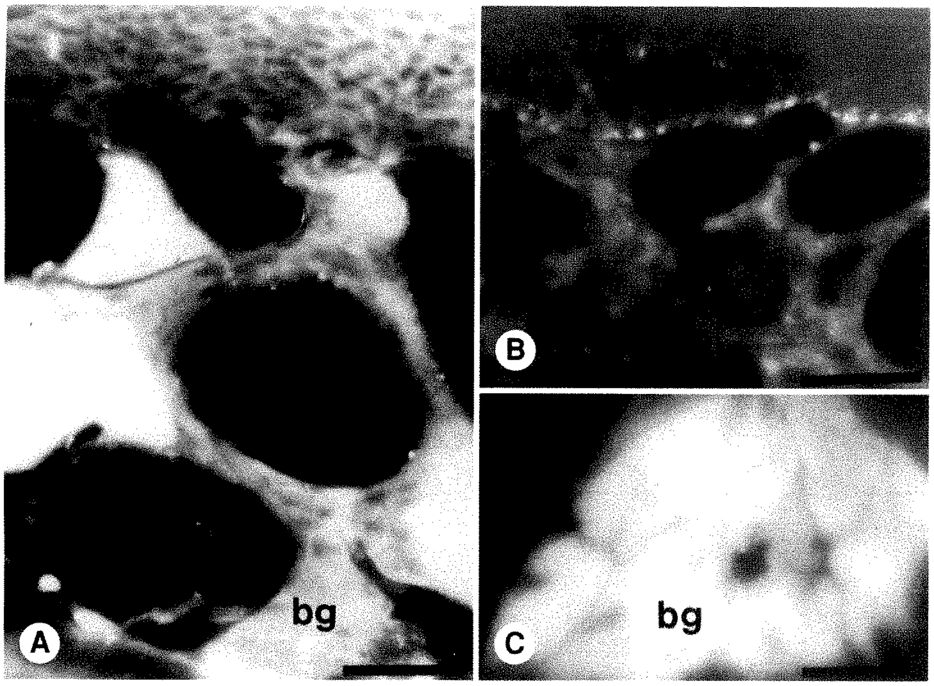

Fig 5. Fluorescein-labeled MR immunoreactivity in Bowman's glands (bg) of mouse. A,C) High-magnification photomicrographs demonstrate immunofluorescence staining for MRs in cytoplasm of Bowman's gland acinar cells. A) Bar — 30 µm. C) Bar — 90 µm. B) In control section in which primary antibody was omitted, there was no immunoreactivity. Bar — 60 µm.

background was noted in the kidney (lane 3). The presence of the OMP product confirms that olfactory mucosa was present in the tissue sample. There was no amplification of the expected bands with olfactory mucosa and kidney samples when reverse transcription was omitted (negative controls), and this finding indicates negligible contribution to the band of interest from spurious sources such as genomic DNA degradation products, primer oligomers, or other contaminating DNA (lanes 4, 5, 8, and 9).

Expression of MR Protein in Olfactory Mucosa. Localization of the protein expression of MR in the olfactory mucosa was sought through immunofluorescence and immunoperoxidase microscopy. At low magnification, immunoreactivity to the synthetic fusion protein, corresponding to 167 amino acids of the N-terminal region of the human renal MR, was

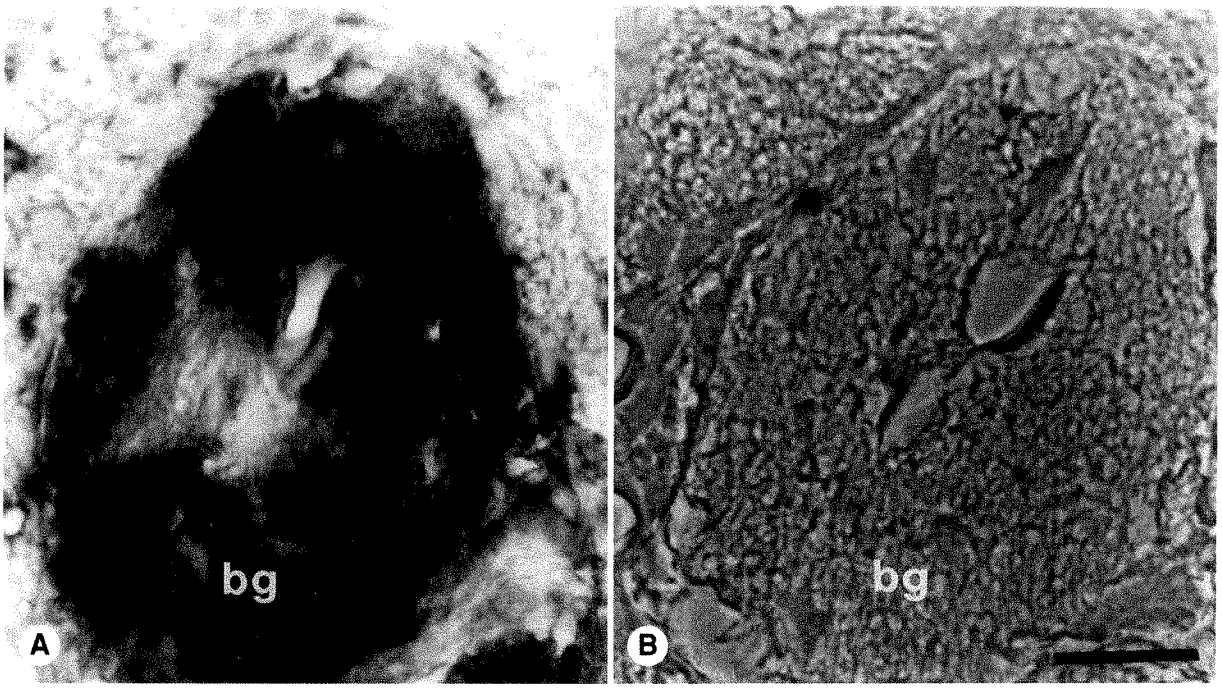

Fig 6. Immunoperoxidase staining of Bowman's glands (bg) in guinea pig. Bar — 10 µm. A) With immunoperoxidase technique, intense immunoreactivity for MRs is clearly demonstrated in cytoplasm of Bowman's gland acinar cells. B) Control micrograph without primary antibody demonstrates no activity.

observed in the olfactory mucosa of the mice (Fig 2A) and guinea pigs (Fig 3A), while activity in neighboring respiratory regions was absent (Fig 2A). When the olfactory mucosa was examined at higher magnification (Fig 4A,B), it became clear that the supranuclear region of sustentacular cells was positive for MRs. Immunoreactivity was also noticed deeper in the epithelium, corresponding to the location of the cell bodies of olfactory receptor neurons (Figs 2C and 4A). Although definitive identification of the cells is not possible at this magnification, these cells appear to be olfactory receptor neurons with immunofluorescence apparent in the perinuclear region and the cellular processes (Figs 2C and 4A). Immunoreactivity in this location was less intense, however, and was not present in all olfactory receptor neurons. In the lamina propria, immunoreactivity was demonstrated in the acinar cells of Bowman's glands and olfactory nerve bundles that consist of bundles of receptor axons surrounded by ensheathing cells (Figs 3A, 5, and 6). While these latter structures demonstrate immunoreactivity for MR, it is not possible to determine whether this represents activity of ensheathing cells or receptor axons. With the omission of the primary antibody, no immunoreactivity was detected (Figs 2B and 3B).

DISCUSSION

Earlier biochemical and histochemical studies identified the presence of MRs, as well as the enzyme 11β-hydroxysteroid dehydrogenase (the specific protector of MR in mineralocorticoid target tissues), in olfactory mucosa.[7,9] The current study provides further evidence for the presence of MRs in the olfactory mucosa by means of RT-PCR. Samples of olfactory mucosa and the known positive control (kidney) demonstrated the presence of mRNA for MRs. By their design, the RT-PCR experiments did not address the question of which cell type(s) within the olfactory mucosa contain the MRs. Therefore, in the present study we utilized techniques that would identify specific target cells containing the MRs by use of polyclonal antibody raised against purified receptor protein. We report that MR-like immunoreactivity is present in olfactory regions, but not respiratory regions, of the mammalian nasal mucosa. The immunoreactivity was seen within the supranuclear regions of sustentacular cells. Less intense immunoreactivity was frequently identified in the perinuclear region and axons of olfactory receptor cells. In the lamina propria, olfactory nerve bundles demonstrated MR immunoreactivity. These structures are located deep within the mucosa and represent bundles of olfactory receptor axons surrounded by ensheathing cells. In addition, acinar cells of Bowman's glands clearly demonstrated intense MR immunoreactivity. In all cell types, activity was primarily localized to the cytoplasm, consistent with both the cellular location of MRs and also earlier studies using this antibody.[15]

Much is known about the biological effects of mineralocorticoid hormones in a variety of tissues. Only recently, however, have studies begun to address the question of the role of mineralocorticoids in olfactory function. Mineralocorticoids and glucocorticoids are the 2 broad classes of corticosteroids secreted by the adrenal glands. Cortisol (in humans and guinea pigs) and corticosterone (in rats and mice), the predominant physiological mammalian glucocorticoids, are believed to regulate cellular growth and development, immune response, electrolyte transport, and other processes. Mineralocorticoids are typically involved in regulation of fluid and electrolyte transport in various epithelial tissues. These hormones act by binding to specific intracellular receptors present within the cells of target tissues. At least 2 types of cytosolic adrenal corticosteroid receptors exist: type I and type II. Type I receptors are referred to as "mineralocorticoid" and type II as "glucocorticoid" on the basis of their in vivo binding selectivity.[6] Historically, mineralocorticoids have been defined as hormones promoting unidirectional transepithelial sodium transport.[5,17] The physiological mineralocorticoid hormone is aldosterone, and its classic action in the nephron is one of sodium retention and potassium secretion at least in part mediated through increases in Na,K-ATPase activity.[18] Besides its renal effects, however, aldosterone has analogous effects on the salivary glands,[19,20] sweat glands,[21] colonic mucosa,[22] amphibian urinary bladder,[23,24] and anuran skin.[25] A coherent physiology for aldosterone involves conservation of sodium in a variety of secretory and absorptive tissues. In these tissues, the enzyme Na,K-ATPase has been localized to the basolateral cell membrane of transporting epithelial cells by cytochemical and autoradiographic techniques.[26] Specific effects of aldosterone on the olfactory mucosa are currently unknown, but it should be noted that Na,K-ATPase is present along the basolateral surface of sustentacular cells and acinar and duct cells of Bowman's glands.[27,28]

Biophysical studies have suggested the presence of a standing short-circuit current across the olfactory mucosa consisting of passive absorption of sodium and other ions across the apical membrane of sustentacular cells.[29] The Na,K-ATPase present along the basolateral surface of these cells actively pumps out sodium into the intercellular space.[27] The current study demonstrates the presence of MRs in sustentacular cells. The physiological action of aldosterone on these cells would, therefore, likely serve to increase the absorption of water and electrolytes across the olfactory mucosa through an increase in Na,K-ATPase activity, and possibly serve to modulate the ion content of the perineural fluid surrounding olfactory receptor neurons. The current study also demonstrates MRs within acinar cells of Bowman's glands. Aldosterone-mediated increases in the activity of Na,K-ATPase in acinar and duct cells of Bowman's glands would likely increase ion resorption, similar to the effects on the kidney and salivary gland.[17-19] The net result would be a decrease in the sodium and chloride content of olfactory mucus. This may have particular significance, given the apparent importance of low mucus chloride concentration in the initial phases of olfactory transduction.[30]

The possible localization of MRs in the olfactory receptor neurons raised the question as to whether the electrical properties of the olfactory neurons could be directly affected by mineralocorticoid hormones. It has been acknowledged for many years that adrenal corticosteroid hormones cross the blood-brain barrier and bind to intracellular receptors in the brain.[31] Moreover, it is worth noting that high levels of MRs have been demonstrated in neurons of the hippocampus.[32,33] Although the role of MRs within neurons is currently uncertain, recent studies have revealed that corticosteroid hormones are able to restore changes in neuronal membrane properties induced by current or neurotransmitters.[33] It is unclear whether these effects are mediated through increases in Na,K-ATPase activity, as seen in epithelial cells. In olfactory neurons, however, high levels of Na,K-ATPase have been reported, particularly on their apical (dendritic) surfaces and receptor cilia.[27,28] Recent studies have demonstrated a relatively high intracellular chloride concentration in these locations that appears to be important for the initial stages of olfactory transduction.[30] The molecular mechanisms responsible for this high intracellular chloride concentration are unknown, but high levels of Na,K-ATPase and sodium, potassium, chloride cotransporter are utilized by other systems.[34] Whether aldosterone increases activity of Na,K-ATPase enzyme in olfactory neurons is currently unknown, but it is possible that high levels of this enzyme mediated by aldosterone could serve to maintain gradients during periods of high ion flux.

Corticosteroids are the most commonly prescribed medications for treatment of clinical olfactory disorders secondary to rhinologic disease. The mechanism of action is unknown, but has been presumed to be secondary to increased airflow to the olfactory cleft mediated by anti-inflammatory effects on the nasal respiratory epithelium.[35] The current report, as well as other recent studies, suggests that corticosteroids may have direct effects on the olfactory mucosa with implications for secretion, absorption, and transduction in the physiological and pathophysiological states.[7,9,35,36] The precise effects of these hormones on the cells of the olfactory mucosa are the subject of current study.

REFERENCES

1. Getchell M, Mellert T. Olfactory mucus secretion. In: Getchell T, Doty R, Bartoshuk L, Snow J, eds. Smell and taste in health and disease. New York, NY: Raven Press, 1991:83-95.

2. Getchell M, Zielinski B, Getchell T. Odorant and autonomic regulation of secretion in the olfactory mucosa. In: Margolis F, Getchell T, eds. Molecular neurobiology of the olfactory system. New York, NY: Plenum, 1988:71-98.

3. Farbman A. Cell biology of olfaction. New York, NY: Cambridge Press, 1992:24-72.

4. Zielinski B, Getchell M, Wenokur R, Getchell T. Ultrastructural localization and identification of adrenergic and cholinergic nerve terminals in the olfactory mucosa. Anat Rec 1989; 225:232-45.

5. Fanestil DD, Park CS. Steroid hormones and the kidney. Annu Rev Physiol 1981;43:637-49.

6. Funder JW. Aldosterone action. Annu Rev Physiol 1993; 55:115-30.

7. Kern RC, Foster JD, Pitovski DZ. Mineralocorticoid (type I) receptors in the olfactory mucosa of the mammal: studies with aldosterone and the anti-mineralocorticoid spironolactone. Chem Senses 1997;22:141-8.

8. Fong K, Kern RC, Pitovski DZ. Regulation of Na,K-ATPase in the mammalian olfactory mucosa by aldosterone [Abstract]. Chem Senses 1997;22:681.

9. Kern RC, Pitovski DZ. Localization of 11β-hydroxysteroid dehydrogenase: specific protector of the mineralocorticoid receptor in mammalian olfactory mucosa. Acta Otolaryngol (Stockh) 1997;117:738-43.

10. DePaolo L, Masoro E. Endocrine hormones in laboratory animals. In: Loeb W, Quimby F, eds. The clinical chemistry of laboratory animals. New York, NY: Pergamon Press, 1989:279-308.

11. Patel PD, Sherman TG, Goldman DJ, Watson SJ. Molecular cloning of a mineralocorticoid (type I) receptor complementary DNA from rat hippocampus. Mol Endocrinol 1989;3:1877-85.

12. Rogers KE, Dasgupta P, Gubler U, Grillo M, Khew-Goodall YS, Margolis FL. Molecular cloning and sequencing of a cDNA for olfactory marker protein. Proc Natl Acad Sci U S A 1987;84:1704-8.

13. Reydon JL, Smith CA. The ultrastructure of normal and otosclerotic human stapes (preliminary studies). Laryngoscope 1968;78:95-121.

14. Arriza JL, Weinberger C, Cerelli G, et al. Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. Science 1987;237:268-75.

15. Krozowski ZS, Rundle SE, Wallace C, et al. Immunolocalization of renal mineralocorticoid receptors with an antiserum against a peptide deduced from the complementary deoxyribonucleic acid sequence. Endocrinology 1989;125:192-8.

16. Fukushima K, Sasano H, Naguro H, Sasaki I, Matsuno S, Krozowski ZS. Immunohistochemical localization of mineralocorticoid receptor in human gut. Tohoku J Exp Med 1991; 165:155-63.

17. Katz A. Renal ATPase: its role in tubular sodium and potassium transport. Am J Physiol 1982;242:207-19.

18. Marver DM, Kokko JP. Renal target sites and the mechanism of action of aldosterone. Miner Electrolyte Metab 1983;9: 1-18.

19. Bundgaard M, Moller M, Poulsen HH. Localization of sodium pump sites in cat salivary glands. J Physiol 1977;273: 339-53.

20. Cossu M, Lantini MS, Puxeddu P, Riva A. Cytochemical localization of ouabain-sensitive, K$^+$-dependent *p*-nitrophenylphosphatase and Ca^{++}-stimulated adenosine triphosphatase activities in human parotid and submandibular glands. Histochemistry 1984;81:221-5.

21. Quinton PM, Tormey JM. Localization of Na$^+$, K$^+$-ATPase sites in the secretory and reabsorptive epithelia of perfused equine sweat glands: a question to the role of the enzyme in secretion. J Membr Biol 1976;29:383-99.

22. Vangesa P, Hopfer U. Localization of alkaline phosphatase and Na$^+$ pump sites in adult rat and rabbit colon by cytochemical and cell fractionation techniques [Abstract]. J Cell Biol 1978;79:238.

23. Crabbe J. Stimulation of active sodium transport by the isolated toad bladder with aldosterone in vitro [Abstract]. J Clin Invest 1961;42:103.

24. Mills JW, Ernst SA. Localization of sodium pump sites in frog urinary bladder. Biochim Biophys Acta 1975;375:268-73.

25. Mills JW, Ernst SA, Dibona DR. Localization of Na$^+$-pump sites in frog skin. J Cell Biol 1977;73:88-110.

26. Ernst S, Mills J. Autoradiographic localization of tritiated ouabain–sensitive sodium pump sites in ion-transporting epithelia. J Histochem Cytochem 1980;28:72-7.

27. Kern RC, Kerr TP, Getchell TV. Ultrastructural localization of Na$^+$/K$^+$-ATPase in rodent olfactory epithelium. Brain Res 1991;546:8-17.

28. Foster JD, Kern RC, Pitovski DZ. Localization of Na, K,ATPase in the mammalian olfactory mucosa [Abstract]. Association for Research in Otolaryngology Abstracts of the Eighteenth Midwinter Research Meeting. 1995:650.

29. Persaud K, DeSimone J, Getchell M, Heck G, Getchell T. Ion transport across the frog olfactory mucosa: the basal and odorant-stimulated states. Biochim Biophys Acta 1987;902:65-79.

30. Reuter D, Zierold K, Schroder W, Frings S. A depolarizing chloride current contributes to chemoelectrical transduction in olfactory sensory neurons in situ. J Neurosci 1998;18: 6623-30.

31. McEwen BS, Jones K, Pfaff D. Hormonal control of sexual behaviour in the female rat: molecular, cellular and neurochemical studies. Biol Reprod 1987;36:37-45.

32. Moguilewsky M, Raynaud J. Evidence for a specific mineralocorticoid receptor in rat pituitary and brain. J Steroid Biochem 1980;12:309-14.

33. Myles K, Funder JW. Type I (mineralocorticoid) receptors in the guinea pig. Am J Physiol 1994;267:268-72.

34. Nauntofte B. Regulation of electrolyte and fluid secretion in salivary acinar cells. Am J Physiol 1992;263:823-37.

35. Robinson AM, Kern RC, Foster JD, Fong KJ, Pitovski DZ. Expression of glucocorticoid receptor mRNA and protein in the olfactory mucosa: physiologic and pathophysiologic changes. Laryngoscope 1998;108:1238-42.

36. Kern RC, Foster JD, Pitovski DZ. Glucocorticoid (type II) receptors in the olfactory mucosa of the guinea pig: RU 28362. Chem Senses 1997;22:313-9.

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COMPARISON OF UPPER ESOPHAGEAL SPHINCTER OPENING IN HEALTHY ASYMPTOMATIC YOUNG AND ELDERLY VOLUNTEERS

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Deglutitive upper esophageal sphincter opening (UES) in the elderly has been incompletely studied. Our aim was to determine in the elderly the temporal and dimensional characteristics of deglutitive UES opening; anterior and superior hyoid and laryngeal excursions as measures of distracting forces imparted on the UES; and hypopharyngeal intrabolus pressure (IBP). Fourteen healthy elderly and 14 healthy young volunteers were studied by concurrent videofluoroscopy and hypopharyngeal manometry during swallowing of 5- and 10-mL barium boluses. The anteroposterior UES diameter, as well as the anterior hyoid bone and laryngeal excursion, was significantly smaller in the elderly compared to the young ($p < .05$) for 5-mL barium boluses, but not for 10-mL boluses. The lateral diameter of UES opening was similar between groups for all boluses. The IBP for 5- and 10-mL swallows in the elderly was significantly higher than that in the young ($p < .05$). We conclude that anteroposterior deglutitive UES opening and hyoid bone and thyroid cartilage anterior excursion are reduced in the elderly. These changes are associated with increased IBP, suggesting a higher pharyngeal outflow resistance in the elderly compared to the young.

KEY WORDS — compliance, hydrodynamic resistance to flow, traction forces.

INTRODUCTION

Information about the effect of aging on upper esophageal sphincter (UES) opening is scarce, and available studies have suggested a reduction of deglutitive UES opening in the elderly compared to the young[1,2] due to a decrease in the distensibility of the cricopharyngeus muscle.[2] Evidence of reduced cricopharyngeus muscle distensibility is that hypopharyngeal intrabolus pressure is significantly higher in the elderly compared to the young,[1,2] the higher pressure being a functional consequence of greater hydrodynamic resistance in the less distensible, elderly UES.

However, opening of the UES during swallowing, in addition to its distensibility, is influenced by the deglutitive relaxation of the cricopharyngeus muscle and the traction forces imparted upon this muscle by contraction of the thyrohyoid and suprahyoid muscles, involved in UES opening.[3-12] Abnormalities of 1 or more of these components may potentially result in reduction in maximum UES opening. The relative contribution of abnormalities of the above-mentioned components to the reduced UES opening reported in the elderly has not been systematically investigated. The aim of the present study was to determine in the elderly 1) the temporal and dimensional characteristics of deglutitive UES opening; 2) the associated biomechanical events, such as anterior and superior hyoid and laryngeal excursions as measures of distracting forces imparted on the UES by contraction of the UES opening muscles; and 3) intrabolus hypopharyngeal pressure as a measure of pharyngeal outflow resistance. We then compared these findings with those in the young.

METHODS

Fourteen young (age 32.8 ± 2.7 years, range 24 to 42 years, 7 male and 7 female) and 14 elderly healthy volunteers without any swallowing difficulties (age 75.0 ± 2.8 years, range 70 to 90 years, 6 male and 8 female) were studied. Studies were approved by the Human Research Review Committee of the Medical College of Wisconsin, and volunteers gave informed consent before their studies.

To determine the anteroposterior diameter and lateral diameter of the UES opening, and the concurrent intrabolus hypopharyngeal pressure, a simultaneous videofluoroscopic and manometric technique was used. For determination of intrabolus hypopharyngeal pressure, a solid-state transducer assembly was used. Two 3-site transducer probes with their pressure sensors 3 cm apart (Gaeltec MMI, Hacken-

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sack, NJ) were secured side-by-side so that the manometric recording sites were separated by 1.5 cm. The transducer assembly was passed transnasally following application of 2% topical Xylocaine (Astra Pharmaceuticals, Westborough, Mass) with a cotton-tipped applicator and positioned such that the most distal site was within the UES high-pressure zone during resting. During swallowing, due to orad excursion, the sphincter climbed onto the transducer above,[1] while the transducer located 1.5 cm more proximally recorded the hypopharyngeal intrabolus pressure during swallowing. Transducers were faced posteriorly. This direction was maintained by the flat shape produced by securing the 2 probes together (4.4 × 2.2 mm). The pressure recordings were referenced to the local atmospheric pressure. All pressure data were recorded on an 8-channel Sensormedics Polygraph and concurrently recorded on a 16-channel Vetter FM tape recorder for subsequent analysis on a computerized digitization system.

The UES dimensions in the anteroposterior direction were measured from the lateral fluoroscopic projections. We measured the narrowest portion of the pharyngoesophageal junction between the level of the true vocal cords and the most proximal part of the esophagus. Upon visual inspection of slow-motion replay of a barium swallow sequence, the maximum dimension of the narrowest portion of the pharyngoesophageal junction was measured.

The computer system for analyzing the manometric data consisted of a 386 IBM PC–compatible computer operating at 33 MHz. This system drives analog-to-digital (A-D) conversion hardware and software (DATAQ Instruments Inc, Toledo, Ohio). The A-D program called CODAS is a menu-driven capture and analysis package that allows for the asynchronous concurrent capture of up to 16 channels of data. The maximum sampling frequency of the system is 50 kHz, which exceeds the frequency requirements for sampling pharyngeal-UES pressure activities. The A-D conversion hardware and CODAS software allowed for capture and analysis of the 6-channel pressure transducer signals at sampling frequencies higher than the limitations of the polygraph recording pens (30 Hz, user's manual, Sensormedics, Inc), as well as analysis of the captured manometric data in time scales unattainable in a conventional polygraph paper-and-pen recording.

For determination of UES anteroposterior and lateral diameters, videofluoroscopy was performed concurrently with manometry. To record the anteroposterior diameter of the UES, subjects sat in a chair positioned laterally to the image intensifier. The subjects were instructed to hold their heads in a neutral position. Subsequently, subjects were turned 90° and anteroposterior recordings were obtained for determination of UES lateral diameter. Videofluoroscopic recordings were obtained at 90 kiloelectron volts with a 9-inch image intensifier and appropriate collimation so that an image was obtained of the posterior mouth, pharynx, and pharyngoesophageal region. Five-milliliter and 10-mL liquid barium boluses (EZ-HD, viscosity 300 centipoise, density 1.88 g/mL; EZ-EM Inc, Westbury, NY) were tested. Each volume was tested 3 times for each projection. Fluoroscopic images were recorded on a Super VHS video cassette recorder (Panasonic AG-1960 Proline, Tokyo, Japan), which recorded 30 frames per 60 fields per second. The manometric and fluoroscopic recordings were synchronized with a specially designed timer (Thalner Electronics, Ann Arbor, Mich). This timer, the video recorder, and the polygraph were electrically connected. The timer generates 2 simultaneous signals. One signal provided a video-displayed time signal in hundredths of a second on the fluoroscopic images, and the other signal generated a 1-Hz, 5-V amplitude timing pulse that was recorded on the chart paper used for manometric recording. The chart paper was run at a speed of 50 mm/s; thus, each millimeter distance on the paper represented 20 milliseconds (ms).

The computer analysis system used for analyzing the videofluoroscopic data consisted of a 486 IBM–compatible computer operating at 33 MHz. The computer system drives an A-D conversion board and program specifically designed for image capture and analysis. The image analysis and capture software (JAVA, Jandel Scientific, San Mateo, Calif) allows capture of standard raster scan video images and morphological analysis of digitized image data. The digitized images are stored as computer files for any subsequent recall or analysis.

All videoradiographic data were analyzed independently by 3 separate observers. Two observers (Kern and Bardan) also participated in performing the studies and were not completely blinded to age group nor tested bolus volume. The third observer who analyzed the radiographic images was blinded to both age groups and tested bolus volumes. Interobserver variability was tested by statistically comparing the findings of the 3 observers.

By means of these techniques, for each swallowed bolus the following were determined: 1) maximum anteroposterior diameter of the narrowest area of UES opening, 2) maximum lateral diameter of the narrowest area of UES opening, 3) total duration of UES anteroposterior opening measured from the onset of its opening, seen by fluoroscopy and heralded by the entry of the bolus head to its return to resting closed state following the passage of the tail of the swallowed bolus, 4) time from the onset of UES anteroposterior opening to its maximum opening, 5) duration that the UES (anteroposterior projection) remained maximally opened, 6) time from the onset of UES closure to its complete closure, 7) hypopharyngeal intrabolus pressure at the time of maximum UES opening at the site immediately proximal to the UES, and 8) maximum superior and anterior excursions of the hyoid bone and the larynx from their resting positions. Distance measurements were corrected for magnification inherent to videofluoroscopy by using the known distance (3 cm) between the manometric recording sites on each catheter assembly.

Statistical analysis was done by 2-way ANOVA. Data in the text are presented as mean ± standard error of the mean unless stated otherwise.

RESULTS

Videofluoroscopic Findings. The videofluoroscopic recordings were analyzed in real time, in slow motion, and frame-by-frame to evaluate the deglutitive UES maximum opening and laryngeal and hyoid bone maximum excursions. No significant interobserver variability was found among the 3 observers.

Deglutitive UES performance was evaluated for dimension and duration of opening. Within-group comparison for the effect of bolus volume on UES opening showed that in both young and elderly, maximum UES anteroposterior diameters for 10-mL barium swallows (13.7 ± 0.6 and 13.0 ± 0.4 mm, respectively) were significantly larger than those for 5-mL barium swallows (12.6 ± 0.6 and 11.0 ± 0.4 mm, respectively; $p < .05$). Among both young and elderly, there was, however, no volume effect on the lateral UES diameter.

Between-group comparison showed that the maximum anteroposterior diameter of the UES opening during 5-mL barium swallows in the elderly (11.0 ± 0.4 mm) was significantly smaller than that in the young (12.6 ± 0.6 mm; $p < .05$). For 10-mL boluses, although UES opening in the elderly was smaller on the average compared to that in the young (13.0 ± 0.4 and 13.7 ± 0.6 mm, respectively), the difference did not reach statistical significance. The maximum lateral UES diameters for 5- and 10-mL boluses in the elderly (21 ± 4 and 22 ± 5 mm, respectively) were similar to those in the young (20 ± 5 and 21 ± 4 mm, respectively).

Within-group comparison showed that the total durations of UES opening, measured from the time of entry of the bolus head into the upper margin of the UES to the time of exit of the bolus tail from this region, for 5- and 10-mL barium boluses were similar in both young (571 ± 8 and 580 ± 9 ms, respectively) and elderly (612 ± 9 and 631 ± 9 ms, respectively).

Between-group comparison showed that the total duration of UES opening in the elderly was significantly longer than that in the young for both 5-mL (612 ± 9 and 571 ± 8 ms, respectively; $p < .05$) and 10-mL (631 ± 9 and 580 ± 9 ms, respectively; $p < .05$) boluses (Fig 1).

The total duration of UES opening comprised 3 distinct intervals: 1) onset of opening to maximum opening, 2) maximum opening to onset of closure, and 3) onset of closure to complete closure. The time interval between the onset of maximum UES opening and the onset of the decrease in its diameter toward closure in the elderly was significantly longer than that in the young for both 5-mL (166 ± 14 and 128 ± 12 ms, respectively; $p < .05$) and 10-mL (155 ± 15 and 124 ± 12 ms, respectively; $p < .05$) swallows (Fig 1).

The intervals between the onset of opening and maximum UES opening, as well as the intervals between onset of UES closure and complete closure, were similar between the 2 groups. However, in both groups the interval between the onset of UES opening and maximum opening was significantly shorter than the interval between the onset of UES closure and complete closure (Fig 1).

Comparison of normalized data between young and elderly also showed that the proportion of time spent in maximum UES opening during swallowing of 5- and 10-mL barium boluses for the elderly (27% ± 2% and 26% ± 3% of the total UES opening duration, respectively) was significantly longer than that for the young (22% ± 2% and 22% ± 2%, respectively; $p < .05$). Again, the time between onset of closure and complete closure in both groups was significantly longer than the time between onset of opening and maximum opening and comprised the largest percentage of the UES opening interval during transsphincteric flow (Fig 2), but like the interval between onset of opening and maximum opening, was similar between the 2 groups.

Within-group comparison showed that the maximum deglutitive anterior excursions of the hyoid and larynx for both 5- and 10-mL boluses were similar in the young group. In the elderly group, the maximum anterior excursion of the hyoid during swallowing of 10-mL boluses was significantly larger than that for 5-mL boluses (18.8 ± 1.7 and 15.0 ± 1.3 mm, respectively; $p < .05$; Fig 3). A similar difference was found for laryngeal anterior excursion for 5- and 10-

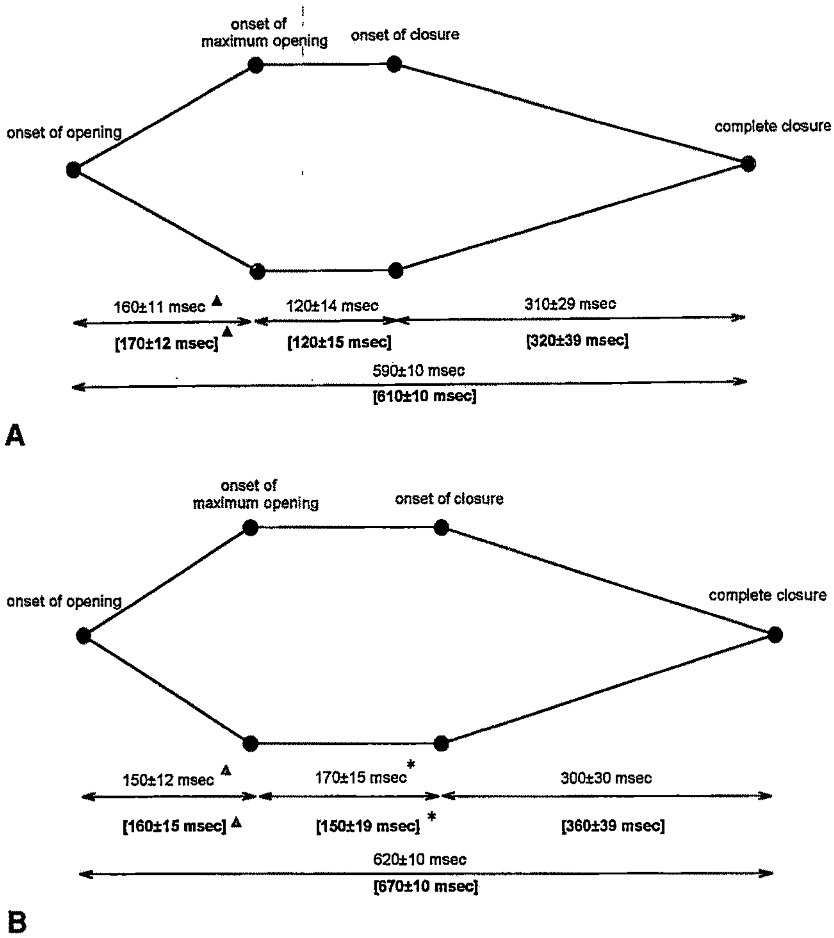

Fig 1. Schematic representation of timing of various stages of upper esophageal sphincter (UES) opening during swallowing of 5-mL and 10-mL barium boluses in A) young and B) elderly. Values for 5-mL swallows are shown above arrow representing various stages. Values for 10 mL are bracketed and shown below arrows. * — between-group comparison of interval between onset of maximum opening and onset of closure ($p < .05$). ▲ — within-group comparison of interval between onset of opening and maximum opening versus interval between onset of closure and complete closure ($p < .05$).

mL boluses in the elderly (10.6 ± 0.6 and 12.3 ± 0.6 mm, respectively; $p < .05$). In both young and elderly groups, the maximum superior excursions of the hyoid bone and larynx did not differ for 5- and 10-mL swallows.

Between-group comparison showed that the average maximum anterior excursions of the hyoid and larynx for 5-mL barium boluses in the elderly (15.0 ± 1.3 and 10.6 ± 0.4 mm, respectively) were significantly smaller than those in the young subjects (20.7

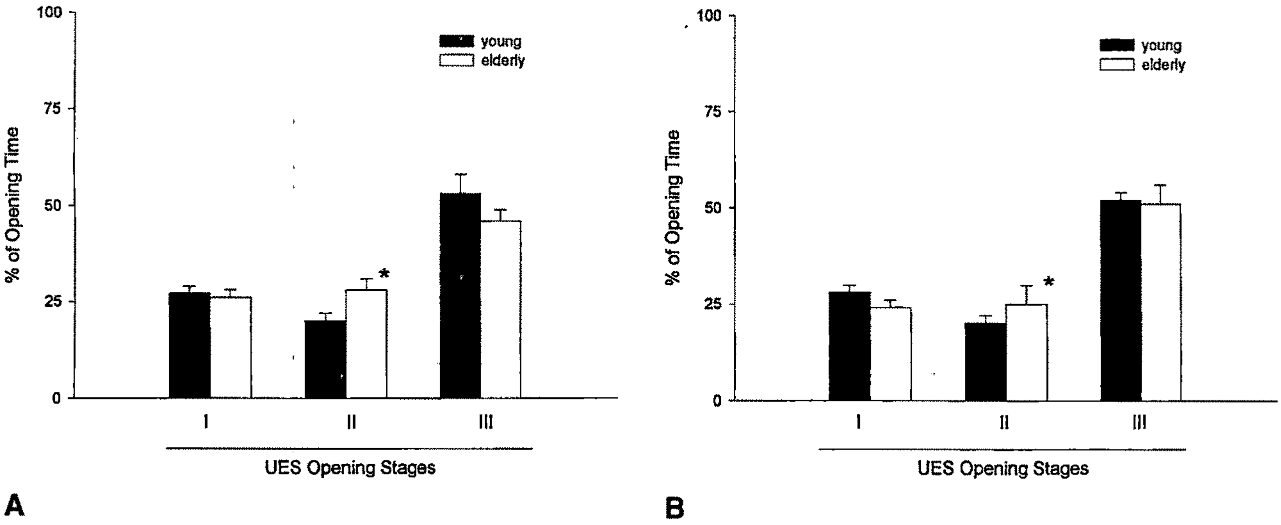

Fig 2. Comparison of normalized data of stages of UES opening duration between young and elderly during swallowing of A) 5-mL and B) 10-mL barium boluses. Stage I — interval between onset of UES opening and maximum opening; stage II — duration that UES remained maximally open; stage III — interval between onset of UES closure and its return to complete closure. As seen, for both 5-mL and 10-mL swallows, duration of stage II in elderly was significantly longer than that in young (* — $p < .05$).

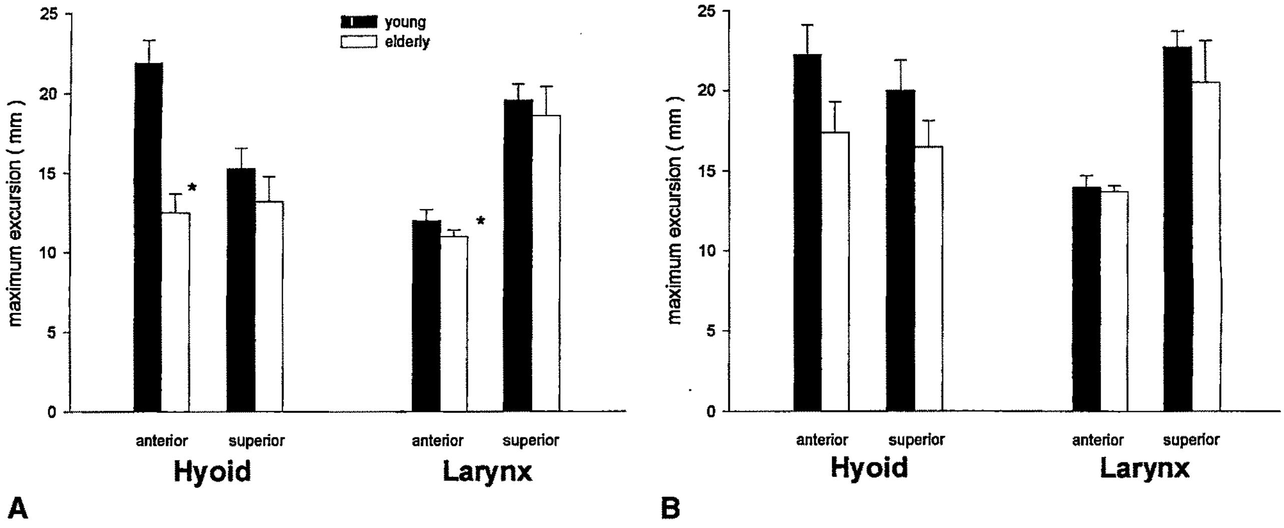

Fig 3. Comparison of maximum anterior and superior hyoid bone and laryngeal excursions during swallowing of A) 5-mL and B) 10-mL barium boluses. For 5-mL barium swallows, maximum anterior excursions of both larynx and hyoid bone were significantly smaller in elderly than in young (* — $p < .05$). During 10-mL barium swallows, these differences did not reach statistical significance. For both 5- and 10-mL barium swallows, superior excursions of larynx and hyoid bone were similar for young and elderly.

± 1.2 and 14.0 ± 0.8 mm, respectively; $p < .05$). For 10-mL boluses, however, the differences did not reach statistical significance (Fig 3). The superior excursions of the hyoid and larynx for both 5 mL and 10 mL were similar in both groups (Fig 3). No gender differences were found in any of the videofluoroscopic measurements for either group.

Manometric Findings. Hypopharyngeal intrabolus pressure was evaluated at the time of maximum UES opening seen in anteroposterior projection. Within-group comparison showed that in both young and elderly, the intrabolus hypopharyngeal pressure during swallowing of 10-mL barium boluses was significantly higher than that of 5-mL boluses ($p < .05$). Between-group comparison showed that for both 5- and 10-mL barium swallows the hypopharyngeal intrabolus pressures in the elderly (14.0 ± 1.5 and 19.5 ± 2.0 mm Hg, respectively) were significantly higher than those of the young (7.2 ± 0.8 and 12.1 ± 0.9 mm Hg, respectively; $p < .05$). There were no gender differences found in the manometric measurements for either group.

DISCUSSION

In this study, the temporal and dimensional characteristics of UES opening during deglutitive transsphincteric flow and associated deglutitive biomechanical events in a group of healthy elderly were determined and compared to similar measurements in a group of healthy young. Study findings indicate that deglutitive UES opening and its associated biomechanical events are significantly different in healthy asymptomatic elderly compared to the young.

Opening of the UES during swallowing is the result of the combined effects of relaxation of the cricopharyngeus muscle (the main component of the UES), traction forces imparted upon this muscle by contraction of the thyrohyoid and suprahyoid muscle groups, and the compliance of the muscular components of the UES.[4,5,7-9,13,14] Abnormalities of 1 or more of these components may result in reduction in UES opening during swallowing and cause incomplete pharyngeal volume clearance and aspiration.

A significant decrease in the maximum cross-sectional area of UES opening during swallowing in a group of healthy elderly volunteers has been reported previously.[2] In that report, the reduction in UES opening in the elderly was found to be due to a smaller transverse sphincter diameter and was attributed to an age-related decrease in sphincter compliance. The present study differs from the above-mentioned report in documenting a significantly smaller anteroposterior diameter of the UES maximum opening in the elderly compared to the young, with no significant difference in lateral diameter when comparing young to elderly. These findings, along with our findings of a reduced anterior excursion of the hyoid bone and larynx, suggest that suboptimal function of the UES opening muscles, in addition to a possible reduction in sphincter compliance, are significant components of altered UES opening in the elderly and indicate that abnormalities of the UES opening are multifactorial.

Similar to what occurs with aging in other striated muscle groups, an age-induced deterioration of the UES opening muscles may result in suboptimal function of these muscles during swallowing. In a recent

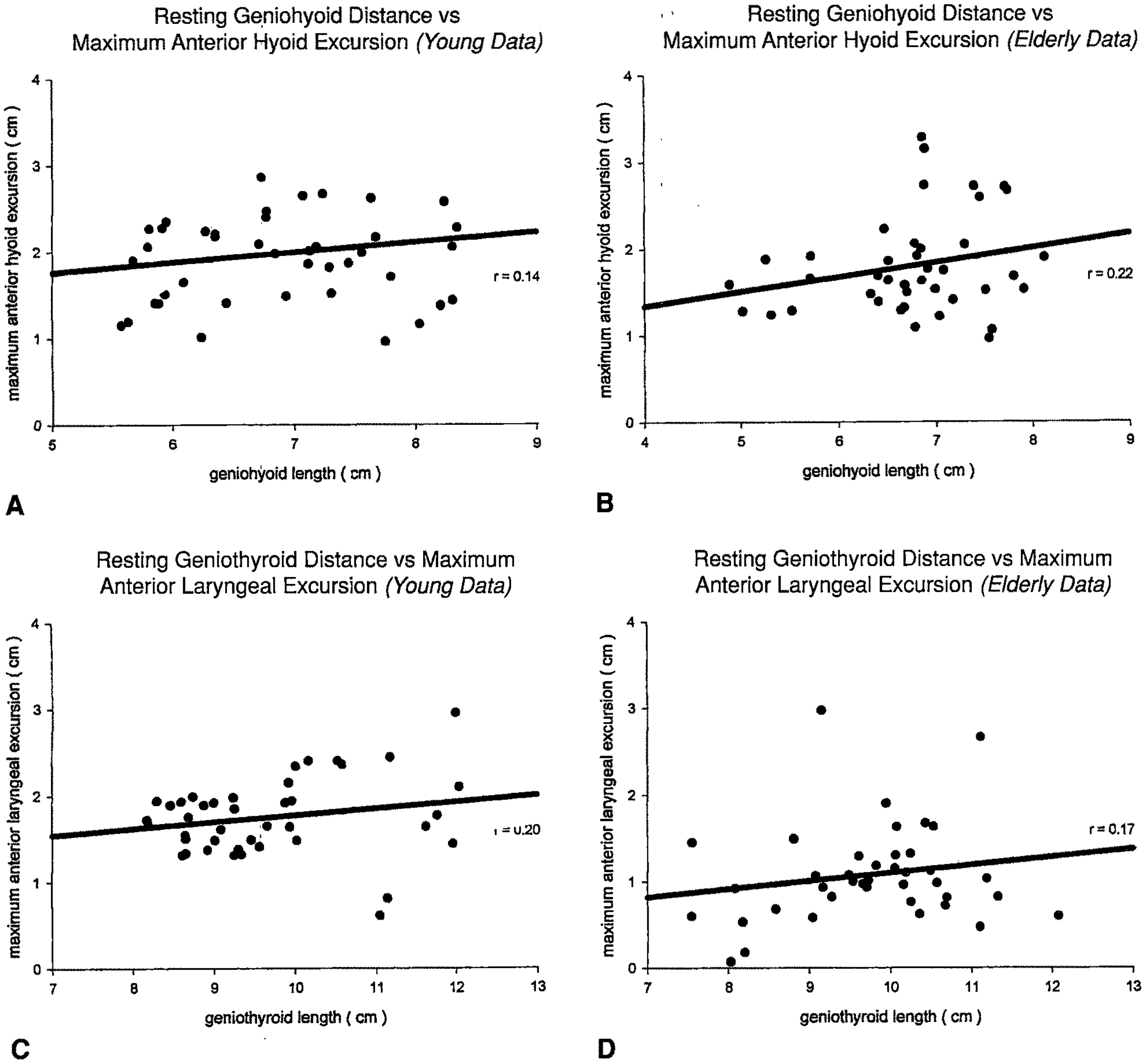

Fig 4. Correlation between A,B) geniohyoid or C,D) geniothyroid resting distance (measure of subject size) and maximum deglutitive anterior A,B) hyoid bone excursion and C,D) laryngeal excursion during swallowing of 5-mL and 10-mL barium boluses. No significant correlation exists for either geniohyoid or geniothyroid resting distance and maximum anterior excursion of hyoid and larynx. A,C) Young subjects. B,D) Elderly subjects.

report, the UES anteroposterior diameter was increased by an isotonic-isometric neck exercise[15] aimed at strengthening these muscle groups,[16] and this outcome supports the notion of suboptimal UES opening muscle function contributing to the smaller UES opening in the elderly.

In both young and elderly groups of the present study, swallowing a larger volume significantly increased the anteroposterior diameter of the UES. The positive effect of increasing volumes of swallowed boluses on the UES opening has been reported previously.[2,4,8,9] These studies of generally young volunteers and with testing of a full range of 2- to 20-mL volumes have shown a positive correlation between bolus volume and UES opening. The present study, in addition to corroborating these findings, shows that this positive modulatory effect is preserved in the elderly. In regard to the finding that the anteroposterior diameter of the UES for 10-mL swallows in the elderly was similar to that of the young, one may consider the above-mentioned modulatory mechanism to be stimulated in the elderly by swallowing of a larger volume, enhancing the contractile activities of the UES opening muscle and resulting in an increased distraction and increased opening of the UES. This notion is supported by our finding that anterior excursion of both the hyoid and larynx was increased significantly by swallowing of 10-mL boluses compared to 5-mL boluses in the elderly.

Absolute differences in distance measurements of the hyoid and larynx for between-group comparisons of anterosuperior excursion can potentially be misleading, due to the possibility of physical scale variability; that is, larger subjects may have the capability of larger hyoid or laryngeal excursion and vice versa. To address this issue, we normalized hyoid and laryngeal excursion in terms of the percent change from a characteristic length parameter indicative of the size of the subject's head. For hyoid excursion, we measured the distance from the anteroinferior corner of the hyoid bone to the posterior-inferior margin of the symphysis menti, as others have reported.[2] This distance represents the approximate length of the geniohyoid muscle. By measuring the geniohyoid length at rest and the geniohyoid length at the time of maximum hyoid anterosuperior excursion, a normalized value for percent change in deglutitive geniohyoid length is calculated as the ratio of geniohyoid length at maximum hyoid excursion to geniohyoid length at rest. Similarly, the characteristic length for laryngeal excursion was measured as the distance from the anterior-superior corner of the air column to the posterior-inferior margin of the symphysis menti, the geniothyroid distance. A comparison of resting geniohyoid length to maximum anterior hyoid excursion and geniothyroid distance to maximum anterior laryngeal excursion (Fig 4) shows that there is no significant correlation between the magnitude of anterior excursion of the hyoid or larynx and the size of the subject as characterized by the geniohyoid and geniothyroid lengths.

Previous studies have reported a significant decrease in UES cross-sectional area when comparing young to elderly subjects.[2] In these studies, as well as the present, the lateral and anteroposterior dimensions were measured for different swallows. Since the lateral and anteroposterior dimensions were not measured concurrently, only an estimate of cross-sectional area can be made from averaged data for each subject. Because of our inability to directly observe concurrently the lateral and anteroposterior dimensions of the UES, we chose not to report as "results" any findings on cross-sectional area. Estimates of cross-sectional area calculated from average lateral and average anteroposterior dimensions yield areas similar to those previously reported.[2] In our study, the significantly smaller area for the elderly compared to the young is attributable to decreased anteroposterior UES diameter, whereas previous studies attributed the decrease to changes in the lateral UES dimension.

Duration of UES opening during swallowing of various boluses has been studied previously, and the existence of a positive volume effect has been reported[4,8,9,17]; however, a similar effect was not found in the current study. This discrepancy could be due to the differences in the range of tested volumes, since only 5 and 10 mL were tested in the present study, and not a full range of 2- to 20-mL boluses, as used in the above-mentioned studies.

A significant increase in bolus transit time through the UES in the elderly has been previously reported.[2] Our study corroborates this finding. In addition, when the duration of UES opening was further divided into its 3 components, namely, interval from onset of opening to maximum opening, duration of maximum opening, and interval from onset of closure to complete closure, it was the duration of maximum opening in the elderly that was found to be significantly longer compared to that of the young for swallows of 5 and 10 mL. In contrast, the other 2 components, namely, the intervals toward maximum opening and toward closure, were similar between the 2 groups.

The finding in the present study of a significantly higher hypopharyngeal intrabolus pressure in the elderly compared to the young is in concordance with previous reports.[1,2] Since previous studies have documented the existence of a positive relationship between resistance to flow and intrabolus pressure,[18,19] this finding is believed to be caused by reduced UES opening and represents a pharyngeal outflow compromise in this age group. The fact that none of these studied subjects had pharyngeal phase dysphagia or hypopharyngeal residue suggests that the above-mentioned outflow compromise has been overcome by compensating mechanisms such as the hypopharyngeal peristaltic pressure wave with higher amplitude and longer duration that has been reported in the elderly compared to the young,[1] as well as the increased intrabolus pressure and longer duration of UES opening in the elderly compared to the young.

In summary, the anteroposterior diameter of deglutitive UES opening in the elderly is significantly smaller than that of the young. This smaller UES anteroposterior diameter is associated with a reduced deglutitive anterior excursion of the larynx and the hyoid bone, suggesting smaller distracting forces imparted by the elderly UES opening muscles on the hyoid bone and larynx. In asymptomatic elderly, the resistive effect of this decrease in UES opening on transsphincteric flow is overcome by an increase in hypopharyngeal intrabolus pressure and longer duration of maximum UES opening. The positive modulatory effect of swallowed bolus volume on UES opening, along with the associated hyoid-thyroid anterior excursion, is preserved in the elderly and may be responsible for the similarity of UES diameter, as well as hyoid-thyroid anterior excursion, between young and elderly during swallowing of larger volumes.

REFERENCES

1. Shaker R, Ren J, Podvrsan B, et al. Effect of aging and bolus variables on pharyngeal and upper esophageal sphincter motor function. Am J Physiol 1993;264:G427-G432.

2. Shaw DW, Cook IJ, Gabb M, et al. Influence of normal aging on oral-pharyngeal and upper esophageal sphincter function during swallowing. Am J Physiol 1995;268:G389-G396.

3. Asoh R, Goyal RK. Manometry and electromyography of the upper esophageal sphincter in opossum. Gastroenterology 1978;74:514-20.

4. Cook IJ, Dodds WJ, Dantas RO, et al. Opening mechanisms of the human upper esophageal sphincter. Am J Physiol 1989;257:G748-G759.

5. Dodds WJ, Stewart ET, Logemann JA. Physiology and radiology of the normal oral and pharyngeal phases of swallowing. Am J Roentgenol 1990;154:953-63.

6. Ekberg O. The normal movements of the hyoid bone during swallow. Invest Radiol 1986;21:408-10.

7. Hellemans J, Pelemans W, Vantrappen G. Pharyngoesophageal swallowing disorders and the pharyngoesophageal sphincter. Med Clin North Am 1981;5:1149-71.

8. Jacob P, Kahrilas PJ, Logemann JA, Shah V, Ha T. Upper esophageal sphincter opening and modulation during swallowing. Gastroenterology 1989;97:1469-78.

9. Kahrilas PJ, Dodds WJ, Dent J, Logemann JA, Shaker R. Upper esophageal sphincter function during deglutition. Gastroenterology 1988;95:52-62.

10. Lund WS. A study of the cricopharyngeal sphincter in man and in the dog. Ann R Coll Surg Engl 1965;37:225-46.

11. Mosher HP. X-ray study of movements of the tongue, epiglottis and hyoid bone in swallowing, followed by a discussion of difficulty in swallowing caused by retropharyngeal diverticulum, postcricoid webs and exostoses of cervical vertebrae. Laryngoscope 1927;37:235-62.

12. Shelton RL Jr, Bosma JF, Sheets BV. Tongue, hyoid and larynx displacement in swallowing and phonation. J Appl Physiol 1960;15:283-8.

13. Lang IM, Shaker R. An update on the physiology of the components of the upper esophageal sphincter. Dysphagia 1994; 9:229-32.

14. Logemann J. Evaluation and treatment of swallowing disorders. San Diego, Calif: College-Hill, 1993.

15. Shaker R, Kern M, Bardan E, et al. Augmentation of deglutitive upper esophageal sphincter opening in the elderly by exercise. Am J Physiol 1997;272:G1518-G1522.

16. Jurell KC, Shaker R, Mazur A, Haig AJ, Wertsch JJ. Effect of exercise on upper esophageal sphincter opening muscles: a spectral analysis [Abstract]. Gastroenterology 1997;112:A757.

17. Cook IJ, Dodds WJ, Dantas RO, et al. Timing of videofluoroscopic, manometric events, and bolus transit during oral and pharyngeal phases of swallowing. Dysphagia 1989;4:8-15.

18. Brasseur JG, Dodds WJ. Interpretation of intraluminal manometric measurements in terms of swallowing mechanics. Dysphagia 1991;6:100-19.

19. Ren J, Massey BT, Dodds WJ, et al. Determinants of intrabolus pressure during esophageal peristaltic bolus transport. Am J Physiol 1993;264:G407-G413.

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PSYCHOSOCIAL ADJUSTMENT AFTER LARYNGEAL CANCER SURGERY

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The objective of the study was to assess the psychosocial adjustment of 111 patients, and 87 partners, after laryngeal cancer surgery. Sixty-nine patients were grouped as having had radical surgery (total or near-total laryngectomy), and 30 as having had functional surgery (horizontal supraglottic laryngectomy or cordectomy). The Psychosocial Adjustment to Illness Scale Self Report questionnaire was used as the primary outcome. No significant differences were found between groups when global adjustment or domain adjustment was compared. Patient and partner responses were almost identical. Work and family relationships were the domains with poorest adjustment for both patients and partners. Information about treatment expectations was negatively rated by functional surgery patients, especially by those who underwent a cordectomy. We conclude that patient perspectives should be considered and consulted to 1) evaluate patient opinion about treatment results, 2) identify patients with special support needs, and 3) inform patients according to other patients' priorities, based on their experience.

KEY WORDS — laryngeal neoplasm, quality of life, speech, surgery.

INTRODUCTION

A permanent tracheal stoma and loss of speech are considered the key sequelae after surgery for cancer of the larynx. Laser, radiotherapy, organ preservation programs, and radical surgery are available options for laryngeal cancer treatment with different expectations and consequences. Most studies consider survival and disease control as the end points of their clinical trials, without providing analysis about the patient's opinion regarding morbidity and the achievement of posttreatment expectations. Selection of treatment involves a trade-off between survival and morbidity; these trade-offs are made by the patient and the physician according to the information given by the latter about treatment options and consequences. This information is usually based more on the physician's opinion about sequelae than on real data coming from patients, although patients are better qualified to inform about their treatment morbidity and outcomes. Quality-of-life studies can give us a better idea and understanding of what patients consider to be their problems and priorities after treatment.

Relatively little is known about the adaptation of patients after treatment, and how the presence of sequelae impacts the different aspects of a patient's personal and family life. Although the concept of quality of life is easily understood, its definition and evaluation tools are not familiar outside the behavioral science area. Evaluation of quality of life in head and neck cancer is especially important, since the treatment sequelae impact directly on basic daily functions (breathing, swallowing, speaking, external appearance). There is a critical need for quality-of-life studies in the head and neck cancer area, in which different treatment options carry very different consequences.[1] Different tools have been used in the analysis of quality of life.[2-5] The need for a standardized quality-of-life assessment tool is evident, in order to compare results between different institutions and studies.[6-8] Since multidimensionality and subjectivity are the 2 main characteristics of quality of life, measurement tools should cover a broad range of content, including physical, emotional, and social well-being.[9] These measurement tools should focus on all areas of life, not just those directly related to the surgical sequelae, and should reflect the patient's opinions.

The purpose of this study was to evaluate the impact of surgical sequelae after laryngeal cancer treatment on the psychosocial adjustment of the patient and his or her family, and to compare psychosocial adjustment according to different surgical operations. Never before has a study of these characteristics been performed in our country, and no objective information regarding our cultural environment was available. The only way to compare results between studies is by using the same or comparable standardized instruments. This process will generate a pool of data that can be used for comparisons between different patient groups with different languages and cultures, an important reference due to the subjectivity of quality of life and the potential influence of specific cultural settings.

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MATERIALS AND METHODS

Study Sample. A questionnaire was administered to patients surgically treated for laryngeal cancer at the Department of Otorhinolaryngology of the Juan Canalejo Hospital (La Coruña, in northwest Spain). Fifty questionnaires were given to patients during routine control visits to the hospital, and 150 questionnaires were sent by mail to patients who had surgery for laryngeal cancer at Juan Canalejo Hospital. Appropriate consent was obtained from patients and partners who were informed, at the hospital or by telephone, of the aim of the study, and assured of the confidentiality of data and the voluntary character of the study. Instructions were given to answer questions with reference to the 4 weeks prior to completion of the questionnaire. Data were collected between March and December 1996. There was no age or diagnosis limitation in patient selection. Follow-up ranged from 8 to 201 months, with an average of 64 months, with 65% of the patients followed more than 36 months. One hundred eleven patients (55%; 111/200), surgically treated for carcinoma of the larynx or hypopharynx, and 87 partners answered the questionnaire. Seventy percent (35/50) of the questionnaires given at the control visit were answered, and 50.6% (76/150) of those sent by mail.

Patients were divided into 2 different groups.

1. Radical surgery: patients who underwent total laryngectomy (TL; 62% or 69/111) or near-total laryngectomy (NT; 11% or 12/111).

2. Functional surgery: patients who underwent a horizontal supraglottic laryngectomy (SG; 17% or 19/111) or a cordectomy (C; 10% or 11/111). No patient in this group had, at the time of the study, a nasogastric or gastrostomy feeding tube, and all had their stomas closed.

Partners were divided into the same groups.

1. Radical surgery: partners of patients who underwent TL (64% or 56/87) or NT (12% or 10/87).

2. Functional surgery: partners of patients who underwent SG (16% or 14/87) or C (8% or 7/87).

The mean age of the patient group was 61.6 years (range 36 to 84), and 99% (110/111) were male. The partners' mean age was 60 years (range 28 to 81), and all were female.

Instrument. Data were collected through the Spanish version of the Psychosocial Adjustment to Illness Scale Self Report (PAIS-SR) questionnaire[5,10] and through the Mayo Clinic Postlaryngectomy Questionnaire[11] (MCPLQ), translated by the senior author (J.H.). The Spanish version of the PAIS-SR is a validated 45-item scale, involving 7 dimensions of adjustment to illness: health beliefs and patient satisfaction (8 items), vocational (6 items), domestic (8 items), social functioning (6 items), sexual relationships (6 items), extended family relationship (5 items), and psychological distress (7 items). Items are rated on a 4-point scale of increasing impact of their illness on adaptation, ranging from 0 to 3. On the PAIS-SR, scale direction is altered on every other item to help reduce position response biases.

The 7 primary PAIS-SR domains were developed through a combination of rational-deductive and empirical-analytic procedures. The domain constructs have been repeatedly identified as having high predictive relevance and clinical utility concerning adjustment to illness. The sum of each item in each domain will give the domain score. The 7 domain scores of the PAIS-SR are summed to generate a PAIS total adjustment score. A respondent's PAIS-SR raw dimension and total adjustment scores are transformed into specific values, designated T scores, through the use of a PAIS normative library and scoring procedure.[10] This transformation will give the value of the T score by domain, and the transformation of the total adjustment score the value of global T score. Because domain T scores represent a "normalizing" transformation, PAIS-SR norms provide a true actuarial assessment of the respondent's status.[10]

The PAIS is not specific for head and neck cancer, and results are referenced to a control group of 114 patients with different types of cancer.[10] This comparison group included patients with lung and breast cancer, lymphoma (Hodgkin's, non-Hodgkin's, and leukemia), and neoplasms of the head and neck other than the larynx. The mean age is 53.1 years, 64% were male, 79% were married, 4% were single, and 17% were divorced. Most patients were treated with surgery and/or chemotherapy. Around 55% of the patients had had metastases at the time of PAIS-SR completion. A T score of 50 is the reference value for the control group; if, in a specific domain, patients with laryngeal cancer have a T score of 50, adjustment is similar to that of the comparison group. A T score higher than 50 reflects an adjustment worse than that of the comparison group. In the head and neck, the PAIS-SR has previously been used in quality-of-life studies in laryngeal cancer patients and maxillectomy patients.[8,11,12]

The MCPLQ[11] is a detailed questionnaire designed by Daniel Rohe and Robert L. Keith, from the Division of Psychology and the Section of Medical Speech Pathology, respectively, from the Mayo Clinic and Mayo Foundation. It comprises 48 questions that provide medical and demographic data; methods used

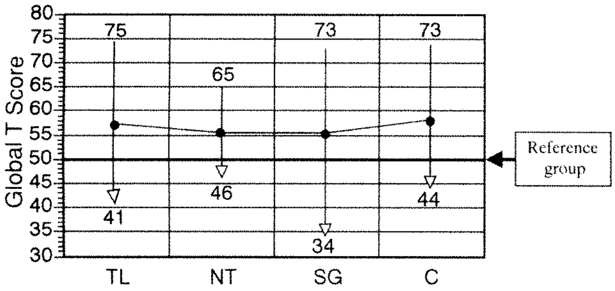

Fig 1. Mean and range global T score by type of surgery performed. Reference group score is 50. TL — total laryngectomy; NT — near-total laryngectomy; SG — supraglotttic laryngectomy; C — cordectomy.

to cope with disability; perception of the treatment process; feelings about the preparation received for treatment; and perceptions of speech quality, swallowing, and overall adjustment to surgical treatment.

Statistical Procedures. The mean scores were calculated for all items and scales. Differences between the means were calculated with the *t*-test for nonparametric samples. Data were analyzed with Stat-View 4.01 (non-FPU) software from Abacus Concepts Inc with an Apple Macintosh LC.

RESULTS

The global T score for the patient groups showed no differences, with scores of 56.44 for the functional group and 56.92 for the radical group. Both groups had an adaptation worse than that of the reference cancer patients group, whose reference T score is 50. No significant differences were found when global adaptation was compared by surgical technique (Fig 1). Adaptation was almost identical in all PAIS domains in both groups of patients (Fig 2[5,10]), with a slightly better but not significantly different adaptation for the functional group in the domains health, work, spouse, family, and psychological. Similar results were found when comparing patients and spouses as a group (Fig 3) or by specific operation performed (Table 1[5,10]).

The worst psychosocial adaptation was found in the domains of social activities and work, and this finding was consistent in patient and spouse groups, independent of type of surgery (Figs 2 and 3). Forty-three percent of patients in the radical group and 25% in the functional group have important problems or are unable to maintain the work they were doing before surgery (Fig 4). When asked how they physically perform their job or daily activities, 45% of the radical group and 40% of the functional group answered not very well or poorly (Fig 5). Statistically significant differences were found in the work and psychological distress domains when comparing patients who went back to work after surgery versus those who did not (Fig 6). Those not going back to work after surgery have a worse adaptation in both domains.

Assessment of the changes in family relationships as a result of treatment shows a difference in patient and spouse groups, for both types of surgery, when compared with the reference group, with almost no dispersion of data (Figs 2 and 3). The reason for this bad adjustment is the lack of help from the close family that the patients seem to have. Around 85% of patients and spouses show no changes in regard to maintaining family relationships, but 96% of patients and 91% of spouses answer they receive not enough help or no help from family members even though they perceived a great need for it (Fig 7). This led to a decrease in interest in family relationships in 86% of the patients and 96% of the spouses.

Better psychological adjustment in social activities was found in the patient group as compared with the control group. Patients maintain the same interest in leisure activities as before surgery in 90% of the cases, and spouses in 87%. Patients undergoing radical surgery show no desire for recreation and social activities at a rate of 12.2%, versus 3.4% of the functional group. This difference is not found between spouses, being 13.5% for the radical group and 10% for the functional group.

Adaptation in the sexual domain is very similar to that of the reference group. Only 5% of the patients

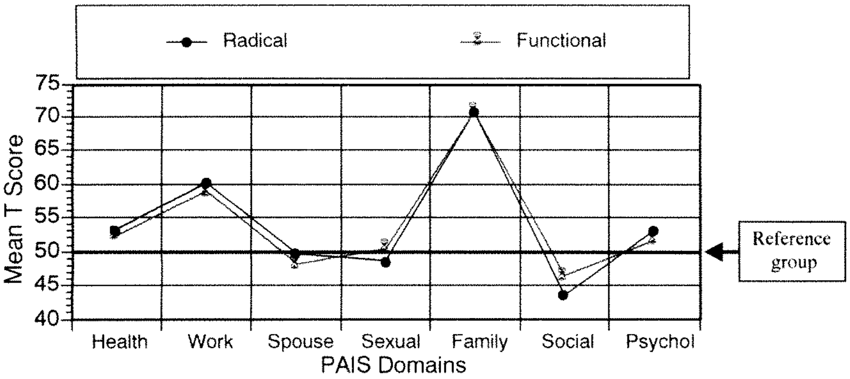

Fig 2. Patient domain T score profile for functional and radical surgery. Reference group score is 50. PAIS — Psychosocial Adjustment to Illness Scale.[5,10]

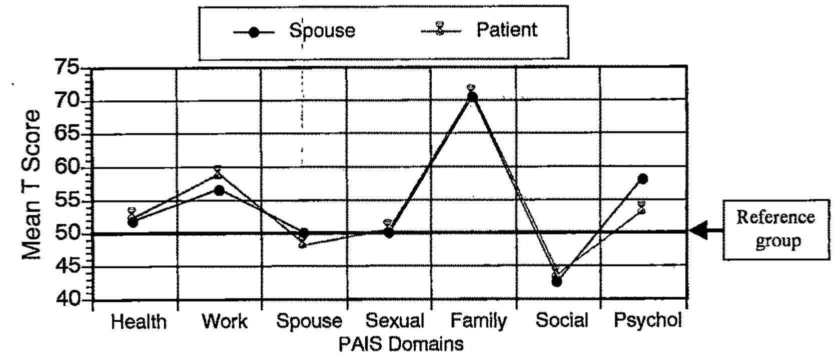

Fig 3. Domain T score profile for spouse and patient. Reference group score is 50.

refer to an important change in their sexual life. Although no statistical differences were found, patients and spouses in the functional surgery group indicate a worse sexual adaptation than the radical surgery group in all sexual areas explored (Table 2).

Patients indicate an increase in anxiety and stress in 65% of the cases, although only 20% of patients identify it as quite a bit or extremely increased. This feeling is higher in the spouse group, in which 78% experience an increase in anxiety, with 30% reporting this increase as moderate or extreme. This same picture is found for temper control, depression, feelings of guilt, self-respect, and appearance. Spouses show a worse adaptation than patients in all domains, and no differences were found between different surgery groups.

When patients were asked about the information given to them about their tumor, 23.5% (26/111) felt it was deficient and would like to have had more details. Information about treatment and posttreatment expectations was poorly rated by 16% of patients, with 7% of the patients saying that treatment was worse than the illness, and that they were not sure it was worthwhile being treated (Fig 8).

DISCUSSION

Options for treatment of laryngeal cancer include therapies that may heavily disrupt patient basic functions (eating, speaking, breathing, swallowing) and compromise a wide range of work, psychological, social, and familial activities. Quality of life is often referred to as an element to be considered in the decision-making for treatment selection. Although the term is frequently used, it is seldom defined and measured.[13] Physicians may have an idea of what treatment and its sequelae may be, but health care professionals' impressions regarding treatment and illness do not fully correlate with patient priorities.[14,15] For this reason, the patient perspective should always be considered when evaluating results of clinical treatment.

Survival and locoregional control are frequent end points of clinical research trials. We feel these end points should be interpreted in the light of quality-of-life data. This would give us an idea whether patients feel that prolongation of survival is worth the treatment and its sequelae, and to what extent pretreatment expectations were achieved. The alternative may be good medical results but an unsatisfactory patient-based outcome. Quality of life can only be described by the individual, and must take into account many aspects of life.[16]

In our study, a permanent stoma and loss of speech are not the critical factors that determine quality of life in patients after surgery for laryngeal cancer. No significant differences were found between patients with a permanent stoma and alaryngeal speech versus patients without these sequelae. The validity of

TABLE 1. COMPARISON OF T SCORES BY PAIS DOMAIN OF PATIENTS AND SPOUSES ACCORDING TO SURGERY PERFORMED

	TL		*NT*		*SG*		*C*	
Domain	*P*	*S*	*P*	*S*	*P*	*S*	*P*	*S*
I	53.91	53.69	49.83	50.00	50.00	49.70	56.45	56.28
II	60.18	56.42	60.75	55.90	58.66	57.28	59.18	54.42
III	50.42	51.12	45.58	45.20	49.77	50.28	45.45	48.71
IV	48.74	49.00	48.00	52.70	49.00	48.42	52.90	51.71
V	70.77	70.62	70.58	70.30	70.77	70.35	70.81	70.71
VI	46.54	43.44	45.50	47.60	41.50	43.85	46.90	37.85
VII	51.47	53.21	53.33	58.00	52.77	57.64	53.90	58.14

PAIS — Psychosocial Adjustment to Illness Scale (see references 5 and 10 for definition of domains); TL — total laryngectomy; NT — near-total laryngectomy; SG — supraglottic laryngectomy; C — cordectomy; P — patients; S — spouses.

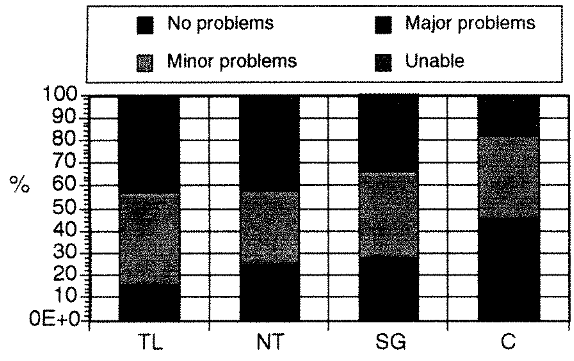

Fig 4. Work problems by type of surgery performed. (Computer program indicates zero value by "0E.")

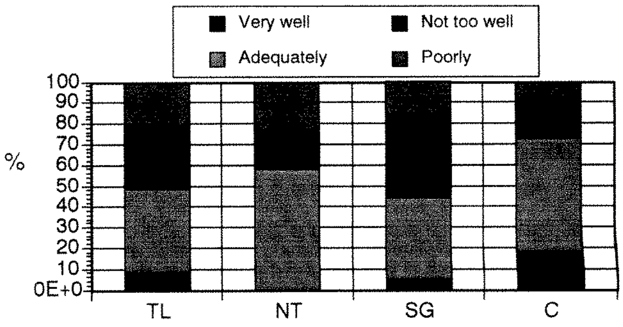

Fig 5. Physical capacity for work by type of surgery performed.

these data is supported by the conformity with the spouse answers. These results agree with other studies on quality of life in laryngeal cancer.[16-20] However, in the studies of DeSanto et al,[8,11] done with a similar patient sample and the same measurement tools, patients undergoing conservation surgery have a significantly better adjustment than patients with total or near-total surgery. The main differences in the sample groups are that only 1 of our patients was female, and none of our patients underwent surgery after irradiation failure. Different populations, different cultural and socioeconomic characteristics, and different availability of health care all influence treatment planning and outcome. There is no doubt that a permanent stoma and alaryngeal speech are serious disabilities, but to what extent this affects patient general and psychological well-being needs a different interpretation. Functional restrictions do not necessarily have a negative impact on quality of life.[20] Seventy-five percent of patients in this study had had their laryngectomy more than 3 years earlier, so most patients had time for coping and adjustment. Longitudinal studies show that time is an important factor in patients' recovery.[20] Patients may learn to cope with treatment sequelae to the extent that they do not interfere with life satisfaction.

An interesting finding is that patients undergoing cordectomy had no better adjustment than patients with more aggressive therapy, and had the worst adjustment in the health domain. This might be due to the fact that patient expectations are not achieved after treatment. Patients with early-stage glottic carcinoma are informed that the disease is curable in a high proportion of cases, with the only negative impact being on voice quality. In our study, almost half of the patients undergoing a cordectomy rated treatment information and treatment preparation as bad or deficient. On the other hand, one third of patients undergoing a radical operation rated the information about treatment and treatment preparation as bad or deficient. As a result of this study, it is obvious that we must reconsider the information given to patients undergoing partial laryngectomy at our institution. Probably, we underestimated the morbidity of cordectomy, putting more emphasis on the fact that the patients were going to avoid a permanent stoma and loss of laryngeal voice. Lack of help from the family is the most negative and constant complaint from all our patients. Social and medical support are important elements in improving patient self-confidence and satisfaction. We must realize that patients and their families face a new and threatening situation when they are informed about cancer and treatment morbidity. Adequate information and training, before and after treatment, for both the patients and close family will reduce anxiety and fears generated by this new situation.[21-23]

Support is necessary for all patients, and especially for those who have tobacco and alcohol habits and come from substandard cultural and socioeconomic environments. Identification of patients in frail psychological condition will alert us to the need for closer support and counseling to reduce the negative

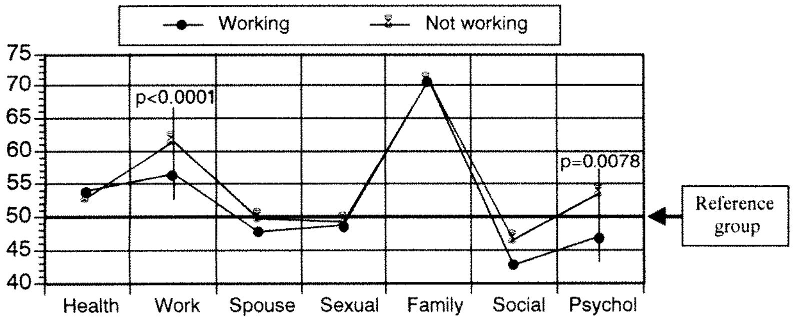

Fig 6. Patient domain T score profile for working status after surgery. Reference group score is 50.

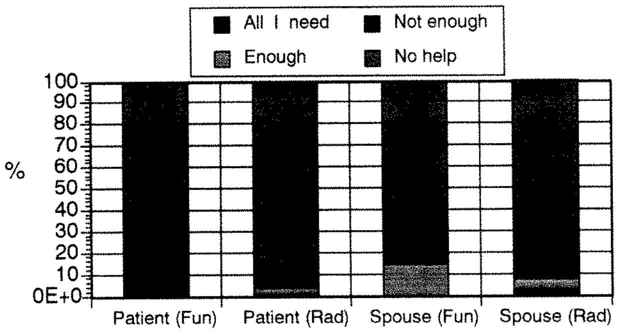

Fig 7. Help received from other family members. Fun — functional surgery; rad — Radical surgery.

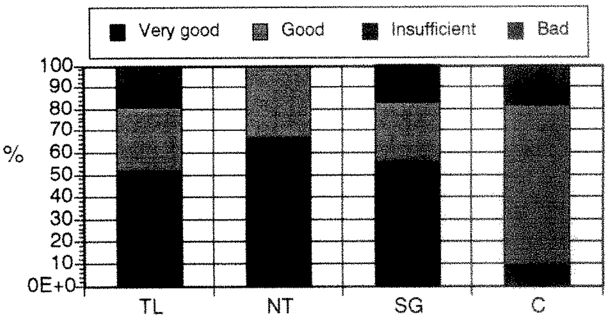

Fig 8. Patient rating of quality of information about treatment expectations by type of surgery performed.

impact that cancer diagnosis and treatment has on patients.

About 75% of our patients and partners confirmed anxiety and depression as a result of treatment. The index of depression is usually higher in the partner group.[21,22] The spouse is usually the closest available assistant to most patients. The lack of adequate instruction and information to the family about possible complications and how to manage them creates a constant state of anxiety, mainly during the first months after treatment.[23] This anxiety is increased by the lack of close family support and no availability of health providers close to the patient's home. Pretreatment information should explain to patients and families the nature of the disease, treatment options, and morbidity and how to cope with it. This information should be given by support groups involving otolaryngologists, nurses, speech therapists, psychologists, social workers, and volunteer patients. In our department it is a priority for the surgeon to conduct a personal interview with each patient before surgery. We appreciate that adequate pretreatment and posttreatment information reduces hospital stay, reduces pain, and increases patient satisfaction.[24-26]

Only 27% of patients undergoing cordectomy and 11% of those undergoing a supraglottic laryngectomy

TABLE 2. CHANGES IN DIFFERENT ASPECTS OF SEXUAL RELATIONSHIP ACCORDING TO SURGERY PERFORMED

Aspect	*Group*	*No Change*	*Slight Change*	*Moderate Change*	*Severe Change*
Interest	Patient				
	Functional	58.62	20.69	10.34	10.34
	Radical	59.75	24.39	12.19	3.65
	Spouse				
	Functional	52.24	17.91	17.91	11.94
	Radical	55.00	25.00	20.00	0.00
Activity	Patient				
	Functional	41.37	34.48	10.34	13.79
	Radical	43.49	40.24	13.41	2.43
	Spouse				
	Functional	30.00	45.00	15.00	10.00
	Radical	40.30	40.30	13.43	5.97
Satisfaction	Patient				
	Functional	51.72	31.03	3.44	13.79
	Radical	52.43	39.02	3.65	4.87
	Spouse				
	Functional	65.00	20.00	10.00	5.00
	Radical	64.18	26.87	2.99	5.97
Ability	Patient				
	Functional	55.17	24.13	10.34	10.34
	Radical	51.22	36.58	10.97	1.22
	Spouse				
	Functional	40.00	40.00	5.00	15.00
	Radical	55.22	34.33	7.46	2.99

Data are percentages of patients and spouses.

went back to work after surgery. Those patients not returning to their work report a significantly worse adaptation in the work domain, of course, and also in the psychological distress domain. Reports from Norway and France show an index of patients going back to work of between 63% and 50%.[27,28] In Spain, the social security system will pay — for life — a salary to those patients undergoing cancer surgery who are unable to maintain their work. This situation may interfere with patients' going back to work, since no patient experiences an important reduction in income or economic problems as a result of treatment.

Adjustment in the sexual domain was very similar to that of the reference group. Only 5% of patients and spouses refer to a major change in the sexual domain as a result of treatment. Even after analyzing each question separately for each group of patients and spouses, no significant differences were found. A higher proportion of substantial changes were found in the patients of DeSanto et al.[8,11] Sexual counseling should be included in support group sessions, to help the couple to overcome minor inconveniences and to make them easier and natural to talk about. Patients appear to desire information and agree to discuss sexual-related problems in a health support setting.[29]

CONCLUSION

Quality-of-life studies illustrate treatment outcome from a patient perspective. Data obtained give first-hand objective information about the results of efforts to improve patient quality of life. This information can improve information and training programs, for pretreatment and posttreatment counseling, according to patient priorities and demands. Identification of patients with critical personal or family conditions determines those requiring a closer follow-up and support before and after treatment. Support group programs must be created, and available in the community, to provide the patient and family with a close setting in which minor problems and fears can be resolved. The utility of these programs should be evaluated with further quality-of-life studies to confirm that these modifications are cost-effective by increasing the quality of life for patients and families.

REFERENCES

1. Morton RP. Quality of life assessment: integral to clinical practice. Clin Otolaryngol 1996;21:1-2.
2. Bjordal K, Elmquist AM, Tolleson E, et al. Development of a European Organization for Research and Treatment of Cancer (EORTC) questionnaire module to be used in quality of life assessments in head and neck cancer patients. Acta Oncol 1994; 33:879-85.
3. Bjordal K, Kaasa S. Psychometric validation of EORTC quality of life questionnaire, 30-item version and a diagnostic specific module for head and neck cancer patients. Acta Oncol 1992;31:311-21.
4. Cella DF, Tulsky DS, Ray G, et al. The functional assessment of cancer therapy: development and validation of the general measure. J Clin Oncol 1993;11:570-9.
5. Derogatis LR. The Psychosocial Adjustment to Illness Scale (PAIS). J Psychosom Res 1986;30:77-91.
6. Aaronson NK. Quality of life research in cancer clinical trials: a need for common rules and language. Oncology (Huntingt) 1990;4:59-66.
7. Aaronson NK, Meyerowitz BE, Bard M, Bloom JR, et al. Quality of life research in oncology. Past achievements and future priorities. Cancer 1991;67:839-43.
8. DeSanto LW, Olsen KD, Perry WC, Rohe DE, Keith RL. Quality of life after treatment of cancer of the larynx. Ann Otol Rhinol Laryngol 1995;104:763-9.
9. Cella DF. Quality of life: concepts and definition. J Pain Symptom Manage 1994;9:186-92.
10. Derogatis LR, Derogatis MA. PAIS & PAIS-SR: administration, scoring & procedure manual II. Baltimore, Md: Clinical Psychometric Research, 1990.
11. DeSanto LW. Cancer of the larynx. Psychosocial aspects. In: Smee R, Bridger GP, eds. Laryngeal cancer. Amsterdam, the Netherlands: Elsevier Science BV, 1994:42-53.
12. Kornblith AB, Zlotolow IA, Gooen J, Huryn JM, et al. Quality of life of maxillectomy patients using an obturator prosthesis. Head Neck 1996;18:323-34.
13. Morton RP. Chemotherapy trials, head and neck cancer, and quality of life. Head Neck 1996;18:92-4.
14. Mohide EA, Archibald SD, Tew M, Young JE, Haines T. Postlaryngectomy quality of life dimensions identified by patients and health care professionals. Am J Surg 1992;14:619-22.
15. Otto RA, Dobie RA, Lawrence V, Sakai C. Impact of a laryngectomy on quality of life: perspective of the patient versus that of the health care provider. Ann Otol Rhinol Laryngol 1997;106:693-9.
16. Morton RP. Laryngeal cancer quality of life and cost effectiveness. Head Neck 1997;19:243-50.
17. Calman KC. Quality of life in cancer patients: an hypothesis. J Med Ethics 1984;10:124-7.
18. Krouse JH, Krouse HJ, Fabian RL. Adaptation to surgery for head and neck cancer. Laryngoscope 1989;99:789-94.
19. Jones E, Lund VJ, Howard DJ, Greenberg MP, McCarthy M. Quality of life of patients treated surgically for head and neck cancer. J Laryngol Otol 1992;106:238-42.
20. List MA, Ritter-Sterr CA, Baker TM, Colangelo LA, et al. Longitudinal assessment of quality of life in laryngeal cancer patients. Head Neck 1996;18:1-10.
21. Mathieson CM, Stam HJ, Scott JP. Psychosocial adjustment after laryngectomy: a review of the literature. J Otolaryngol 1990;19:332-6.
22. Blood GW, Simpson KC, Dineen M, Kauffman SM, Rai-

mondi SC. Spouses of individuals with laryngeal cancer: caregiver strain and burden. J Commun Disord 1994;27:19-35.

23. Wallace K. Communication variables in the design of pre-surgical adjustment to and recovery from surgery. Br J Med Psychol 1986;59:253-61.

24. Hathaway D. Effects of pre-operative instruction on post-operative outcomes: a meta analysis. Nurs Res 1986;35:269-75.

25. Depondt J, Gehanno P. Laryngectomized patients: education and follow up. Patient Educ Couns 1995;26:33-6.

26. Renner MJ. Counseling laryngectomees and families. Semin Speech Lang 1995;16:215-20.

27. Natvig K. Study No. 4: Social, occupational and personal factors related to vocational rehabilitation. J Otolaryngol 1983; 12:370-6.

28. Schraub S, Bontemps P, Mercier M, Barthod L, Fournier J. Surveillance and rehabilitation of cancers of upper respiratory and digestive tracts. Rev Prat 1995;45:861-4.

29. Kaiser FE. Sexual function and the older cancer patient. Oncology (Huntingt) 1992;6(suppl 2):112-8.

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MANAGEMENT OF N0 NECK IN T1-T2 UNILATERAL SUPRAGLOTTIC CANCER

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Early-stage supraglottic cancers (stage I and II) are treated with several different programs. Previously reported data have led us to design a therapeutic protocol in treatment of patients with early-stage squamous cell carcinoma of the supraglottic larynx. From 1991 to 1996, 39 patients with unilateral supraglottic carcinoma were treated according to this protocol. All patients underwent unilateral functional neck dissection and resection of the primary carcinoma in an en bloc fashion. Histopathologic studies showed that 9 (23%) of them had positive nodes, and they received planned adjuvant radiotherapy. None of the 30 patients with histopathologically N0 necks received either adjuvant irradiation or contralateral neck dissection. The mean follow-up period was 34 months. All patients are alive, and none have developed any recurrence in either dissected or undissected sides of the neck. This treatment policy seems satisfactory and will avoid unnecessary therapeutic interventions. Routine bilateral neck dissection may not be necessary in the surgical treatment of all supraglottic laryngeal cancers.

KEY WORDS — early stage of cancer, neck dissection, supraglottic cancer.

INTRODUCTION

The treatment of early-stage squamous cell carcinoma of the supraglottic larynx remains unsettled. Controversy has often focused on whether to treat the primary tumor surgically or by radiotherapy (XRT). However, there are proponents of combined sequential therapy with XRT before or after operation. In fact, all modalities appear to be excellent in controlling early-stage primary tumors.[1-5]

The surgical management of the primary tumor has evolved over the last few decades. Supraglottic laryngectomy may be defined as a turning point in surgery of laryngeal cancer. Classic, extended, and modified supraglottic laryngectomies have provided flexibility and relative safety for both patients and surgeons. Another turning point was functional neck dissection (FND). It completes the conservative trend of supraglottic laryngectomy. The value of FND in high-risk patients is clear from the available data.[1] Thus, traditional total laryngectomy, often combined with radical neck dissection, which was the only accepted surgical therapy until 1950, has changed to these more conservative procedures. Both supraglottic laryngectomies and FND have their own particular indications, and both of them are relatively safe and effective from the point of view of oncology.

Supraglottic cancers have a strong predilection for cervical metastasis. This usually results from simultaneous invasion of the preepiglottic and/or paraglottic spaces. The lymphatic canals within these compartments facilitate metastases to the neck. Patients who present with supraglottic cancer and metastatic neck disease have a worse prognosis than those who are staged as N0.[6,7] In a high percentage, failure of the initial treatment is related to failure in the neck, and many of these patients will die.[6-9]

There is no doubt that patients with neck disease should be treated, and most surgeons prefer to perform a neck dissection in this situation. Management of the N0 neck is more complex, and the challenge is primarily focused on those patients who do not have clinically palpable lymph nodes at presentation. N0 disease can be treated effectively with XRT alone[2,10,11] or with surgery alone.[1,12,13] It is not necessary to treat an N0 neck with a combination of these modalities. An ideal and generally accepted approach is to treat the N0 neck with selected initial therapy for the primary lesion.[11] An elective neck dissection can act as a diagnostic maneuver and clarifies the clinical stage of the tumor, the probability of subclinical neck disease, and the necessity of adjuvant therapy. Presently, there are still arguments on elective neck dissection. Some offer bilateral neck dissection as a routine in all supraglottic cancers.[1,6,14,15] Some consider that elective neck dissection has no significant effect on either the risk of subsequent development of ipsilateral cervical adenopathy or on survival, and they adopt a wait-and-see attitude.[16,17] Others prefer to perform an ipsilateral neck dissection on the side under a higher risk of metastases according to primary tumor site and extent.[18,19]

This study was undertaken to evaluate the role of elective neck dissection in the surgical treatment of

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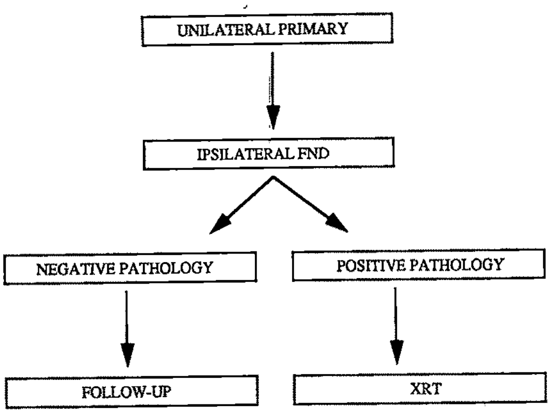

Algorithm for surgical treatment of N0 patients with T1 and T2 supraglottic cancer. FND — functional neck dissection, XRT — radiotherapy.

supraglottic carcinomas. Also, an attempt was made to ascertain whether bilateral dissection is a necessity in the management of all supraglottic laryngeal cancers.

MATERIAL AND METHODS

At our institution we designed an algorithm for management of the N0 neck in surgically treated patients with T1 and T2 supraglottic laryngeal cancers. This is summarized in the Figure. From 1991 to 1996, 39 patients with unilateral supraglottic carcinoma were treated according to this protocol at the head and neck surgery department of Erciyes University.

"Unilateral" lesions of the epilarynx included those of the false vocal cord, aryepiglottic fold, and medial wall of the pyriform sinus. Lesions reaching but not crossing the midline were also included. Tumors located centrally, showing a horseshoe pattern, and tumors with extension outside the larynx involving the tongue base or pyriform sinus were excluded. Clinical assessment of the primary tumor was accomplished by indirect mirror examination, telescopic endovisual photography, direct laryngoscopy, ultrasonography (US), and computed tomography (CT), and tumors were classified according to the staging system recommended by the American Joint Committee on Cancer (AJCC).[20]

Since this study involves the role of elective neck dissection in N0 necks, all patients with positive nodes were also excluded. Only clinically N0 patients were included. If a patient clinically had no palpable nodes but the US or CT scan indicated abnormal lymph nodes, the patient was reexamined. If reexamination revealed no abnormal lymph nodes, then the patient was still staged N0. No patients were staged N+ on the basis of US or CT alone.

The surgical procedure began with unilateral FND and proceeded to supraglottic, extended supraglottic, or near-total laryngectomy. The FND was always performed on the side in which the tumor was in the greatest amount. The lymphatics of zones II, III, IV, and V were removed. The principles of the surgery included removal of the primary tumor with negative margins in continuity with the preepiglottic space and with the lymphatic channels through the thyrohyoid membrane.

All histopathologically N+ patients were given planned postoperative XRT. After surgery these patients received a dose of 50 Gy to both sides of the neck over a period of 5 weeks. None of the histopathologically N0 patients received either adjuvant XRT or contralateral neck dissection. All had at least 14 months of follow-up.

RESULTS

The age of these selected patients ranged between 46 and 68 years (mean 60 years). All patients were male. Of the 39 primary lesions, 8 were classified as T1 and 31 were classified as T2, according to the staging system recommended by the AJCC.

Of the 39 patients who had an elective ipsilateral FND, 30 (77%) were found to be N0 and 9 (23%) were found to have metastases in the cervical lymph nodes histopathologically. All 30 patients who were not given any additional therapy and the 9 who received planned XRT were followed up regularly. The mean follow-up was 34 months (minimum 14 months, maximum 60 months). At present, all patients are alive, and none have developed any recurrence in either the dissected or the undissected side of the neck.

DISCUSSION

One of the most important dilemmas in dealing with laryngeal cancer is the management of necks with N0 disease. The abundant lymphatics of the supraglottic area raise the question of treatment of the neck. A number of patients with clinical N0 disease do have metastatic invasion of regional lymph nodes at the time of surgery. Undertreating these patients will decrease their chances of survival, because the highest probability of survival is always obtained in the first therapeutic approach.[1,11] Therefore, most head and neck surgeons prefer prophylactic treatment of the N0 neck of a patient with supraglottic laryngeal carcinoma. Controversy exists related to the most effective way to manage potential cervical metastases. Two methods are widely accepted in the management of the neck with N0 disease: primary XRT and FND. When used in N0 cases, both modalities are directed to the 20% to 30% of patients with occult neck disease. Some object that this approach leads to overtreatment in 70% to 80% of cases, and a wait-and-see concept is often preferred by these authorities. Shah and Tollefsen[5] did not recommend routine elective neck dissection in patients with endolaryngeal or exolaryngeal supraglottic carcinomas. Coates and Devine[17] observed that only 2 of 8 patients with primary T2 tumors required a delayed neck dissection, and none of the patients with stage I or II supraglottic cancer who did not have a simultaneous neck dissection died of cancer. Soto Varela et al[16] reported that elective neck dissection in N0 patients with supraglottic carcinomas had no significant outcome on either the risk of subsequent development of ipsilateral cervical adenopathy or on survival. They considered that elective neck dissection is not beneficial as a routine strategy for the management of carcinomas affecting the supraglottic larynx.

The only way to avoid undertreatment or overtreatment is by knowing which patients with N0 disease have neck disease at the time of surgery. Unfortunately, at present no preoperative test is available to ascertain the presence of metastases in patients with an N0 neck.[5,9,13,21,22] Noninvasive identification of microscopic deposits of metastatic cancer in the cervical lymph nodes of patients with N0 disease may become possible in the future. Until this is realized, elective neck XRT or elective neck dissection appear to be reasonable when weighed against the risk of neck relapse in patients with an N0 neck. Ramadan and Allen[23] concluded that elective neck dissection decreases neck relapse significantly in patients treated for N0 supraglottic carcinoma. While the relapse rate was 14.3% in patients treated with neck dissection, relapse in the patients who did not have neck dissection was 40.5%. Radiotherapy did not seem to make a significant difference in relapse rate.

Surgery provides not only a treatment, but also a chance for staging the disease. It also determines whether or not an adjuvant therapy is required. In such a situation, some prefer FND over any other surgical approach, because it is the only dissection that removes all of the principal nodal groups in the neck, preserving the 11th nerve, the internal jugular vein, the submandibular gland, and the sternocleidomastoid muscle. Moreover, FND enables a bilateral operation with little or no resultant functional disability and is an oncologically acceptable procedure. When surgery is decided on as initial treatment, then the question arises as to whether an elective neck dissection should be performed routinely in high-risk cases, and if it is done, whether or not a curative neck dissection should be directed to both sides. Both the high probability of occult disease in the neck and the advantages of FND call for bilateral elective neck dissection in patients with supraglottic cancer. Proponents of this approach suggest that this will provide not only cure but also staging and prophylaxis.[6,13,15,24] According to these authors, embryologically, the supraglottis does not form by fusion of 2 lateral masses; therefore, it may be thought of as a midline structure. Further, the preepiglottic space, which contains rich lymphatics, is easily invaded by supraglottic tumors, and this also facilitates bilateral spreading.[1,25,26]

Bocca et al,[13] on the basis of their observations, proposed bilateral neck dissection in all patients with supraglottic cancer, since patients who required a delayed dissection had a lesser probability of surviving than patients who had undergone bilateral dissection at the initial treatment.

In a retrospective review of 202 patients with supraglottic carcinoma who were treated with surgery alone, recurrent metastatic disease to the contralateral, unoperated side was encountered in 19 (19%) of 102 patients. The risk to the contralateral neck was not different between the patients with lateral lesions and patients with epiglottic (midline) lesions. However, the patients with aryepiglottic fold lesions presented with more advanced primaries than the patients with epiglottic lesions. So, the authors decided to perform bilateral neck dissection routinely in all patients with supraglottic carcinoma, both for staging the disease more accurately and for eliminating the possible risk to the contralateral neck.[6] From the same institution, Weber et al[14] concluded that bilateral neck dissection did not increase morbidity and had the potential benefit of avoiding XRT.

In the study of Suarez et al,[18] FND was always performed on the side with clinically positive nodes or on the N0 neck side that is at higher risk of metastasis according to the primary tumor side and extent. The difference between the recurrence rates in the dissected side (2%) and the undissected side (13%) was statistically significant. A similar rate of contralateral recurrences in the undissected side (10%) was observed in N3 cases subjected to radical neck dissection.

Ogura et al[4] noticed that in surgically treated patients the most common site for failure was the neck (21%). To prevent this, they offered preoperative XRT ranging from 40 to 45 Gy in patients selected for conservation surgery. Levendag and Vikram[11] found a 32% incidence of cervical metastases in stage I and stage II supraglottic cancers. Among these, 5 (of 10) relapsed in the ipsilateral neck. In addition, of 21 patients whose neck dissection was negative, 4 (19%) subsequently failed on the contralateral side of the neck. Forty-eight patients had no elective neck dissection, and 14 of them (29%) subsequently relapsed in the neck. Thus, of the 79 patients, 28 (35%) developed cervical nodal metastases. The median time to relapse was 12 months. Although the local recurrence rate in the XRT-treated group is substantially higher, the authors stated that elective XRT to both sides of the neck was the preferable treatment. Marks et al[3] reported that the major site of failure, after low-dose preoperative XRT and conservation surgery for supraglottic laryngeal carcinoma, was the neck. Failures occurred at a rate of 25% in the ipsilateral neck and about 75% in the contralateral neck. The appearance of tumor in the clinically negative neck with an incidence of 75% seems an interesting finding. In another study of Marks et al,[27] the risk of contralateral neck involvement was not found to be related to the size of the primary lesion. They noticed a 7% incidence of contralateral neck metastases for central supraglottic cancers and a close association between the contralateral neck metastases and the presence of palpable lymph nodes or occult lymphatic metastases in the ipsilateral neck. They suggested that the existence of clinical or microscopic ipsilateral metastases was the most reliable indicator of contralateral metastases.

The observation of DeSanto et al[12] was that if dissection on 1 side yields a negative specimen on pathological study, little seems to be gained by performing a contralateral neck dissection. Whereas 33% of those who had positive neck nodes in histopathologic studies on the first side required a dissection on the second side, only 1 of the 98 patients who had a negative specimen on 1 side required second-side dissection. Considering that the incidence of contralateral failure is relatively low with a pathologically N0 ipsilateral neck, DeSanto[7] suggested that dissection of the contralateral neck should be undertaken when intraoperative findings revealed metastatic disease.

The lymphatic drainage of the supraglottic larynx is well documented. The area of the larynx above the vocal cords is drained by the superior group of vessels, which pierces the thyrohyoid membrane, to end in the upper deep cervical nodes. A sharp boundary along the inferior edge of the false vocal cord has been demonstrated.[28] On the other hand, the lymphatic vessels of the larynx form a 2-part, highly compartmentalized system: superficial and deep. The superficial (intramucosal) system communicates freely between the right and left sides of the larynx. The deep (submucosal) system is highly specialized and the most critical for laryngeal tumor spread. There is little or no deep lymphatic communication between the right and left halves of the larynx. Thus, the lymphatic vessels of the supraglottic larynx are divided into right and left groups.[29] So, the pattern of laryngeal lymphatic drainage is mostly unilateral, and contralateral spread without ipsilateral spread is not common.[30] Welsh at al[31] concluded that the direction of lymph flow is an ipsilateral phenomenon and that the anatomic midline appears to direct the course of the flow. However, when the ipsilateral drainage is obstructed by neoplastic infiltration, surgery, inflammatory scarring, or XRT, the endolaryngeal lymphatics continue to function by altering the direction of flow into lower-resistance channels and result in contralateral or bilateral spreading. The concept of treatment of cervical lymph nodes in supraglottic carcinomas closely relates to this basic information. The principles of supraglottic laryngectomy include removal of the supraglottic area in continuity with the preepiglottic space and with the lymphatic channels through the thyrohyoid membrane. This membrane, with its associated lymphatic channels, serves as the point of attachment of the neck dissection. Therefore, supraglottic laryngectomy with neck dissection follows the concept of en bloc resection of the primary tumor with its associated lymphatic channels and lymph nodes.[28]

Cancers of the supraglottic area exhibit a 21% to 32% metastatic rate, and the incidence of bilateral palpable cervical nodes in patients with supraglottic lesions is 5%. The incidence of late contralateral metastasis if ipsilateral nodes are histologically positive is 33%.[28] Our results revealing 23% occult neck disease in the ipsilateral side of the primary lesion suggest that elective neck dissection is necessary in the management of patients with supraglottic carcinoma. If surgical treatment is decided on, an elective neck dissection is warranted on the more suspicious side. This study also showed that the patients who did not have pathologically proven metastases on the side of the neck at the time of primary surgery were unlikely to develop contralateral neck metastases. So, an elective treatment on the second sides of these patients seemed unnecessary. It is our opinion that if the first specimen is truly negative, one can abandon the second-side dissection with relative safety in a patient with unilateral early supraglottic laryngeal cancer. In addition, performing an elective neck dissection on the contralateral N0 neck solely for staging purposes does not seem meaningful. On the other hand, the use of elective XRT as a substitute for elective neck dissection on the first side is less logical. In patients with negative ipsilateral and contralateral necks, it would be unnecessary, and the staging could not be obtained. Preferential FND provided not only cure, but also staging of the tumor more precisely. It also prevents overtreatment of patients with XRT or contralateral neck dissection. However, bilateral elective neck dissection has its place. It seems desirable in patients with clinically determined bilateral lymphadenopathy. Bilateral dissection also is appropriate when the primary tumor is in the midline and the probability of occult metastases is likely. In such cases, delay of the second dissection may increase the rate of failure. If the patient's general health allows, it is oncologically safer to perform bilateral FND simultaneously in these situations.

After FND, recurrences in patients with N0 necks may be explained by intraoperative seeding of cancer cells circulating within the lymphatic vessels. Discontinuous resection of the primary tumor and its associated lymphatics may lead to seeding, obstruction, and misdirection of the lymphatic flow, resulting in contralateral spreading. Therefore, en bloc resection of both lymphatics and the primary lesion in continuity is probably the key point for success. Preoperative XRT may also cause obstruction and misdirection of the lymphatic channels. Our data revealed that postoperative XRT given bilaterally seems to be suitable for patients who had positive ipsilateral neck disease, since no recurrence was encountered in the follow-up period.

We believe that this treatment policy will avoid unnecessary neck dissection and XRT in patients in whom development of contralateral neck metastases is unlikely.

REFERENCES

1. Bocca E. Surgical management of supraglottic cancer and its lymph node metastases in a conservative perspective. Ann Otol Rhinol Laryngol 1991;100:261-7.

2. Nadol JB Jr. Treatment of carcinoma of the epiglottis. Ann Otol Rhinol Laryngol 1981;90:442-8.

3. Marks JE, Freeman RB, Lee F, Ogura JH. Carcinoma of the supraglottic larynx. AJR Am J Roentgenol 1979;132:255-60.

4. Ogura JH, Marks JE, Freeman RB. Results of conservation surgery for cancer of the supraglottic and pyriform sinus. Laryngoscope 1980;90:591-600.

5. Shah JP, Tollefsen HR. Epidermoid carcinoma of the supraglottic larynx. Role of neck dissection in initial surgical treatment. Am J Surg 1974;128:494-9.

6. Lutz CK, Johnson JT, Wagner RL, Myers EN. Supraglottic carcinoma: patterns of recurrence. Ann Otol Rhinol Laryngol 1990;99:12-7.

7. DeSanto LW. Early supraglottic cancer. Ann Otol Rhinol Laryngol 1990;99:593-7.

8. Levendag P, Sessions R, Vikram B, et al. The problem of neck relapse in early stage supraglottic larynx cancer. Cancer 1989;63:345-8.

9. Kligerman J, Olivatto LO, Lima RA, et al. Elective neck dissection in the treatment of T3/T4 N0 squamous cell carcinoma of the larynx. Am J Surg 1995;170:436-9.

10. Spaulding CA, Krochak RJ, Hahn SS, Constable WC. Radiotherapeutic management of cancer of the supraglottis. Int J Radiat Oncol Biol Phys 1988;14:451-4.

11. Levendag P, Vikram B. The problem of neck relapse in early stage supraglottic cancer. Results of different treatment modalities for the clinically negative neck. Int J Radiat Oncol Biol Phys 1987;13:1621-4.

12. DeSanto LW, Magrina C, O'Fallon WM. The "second" side of the neck in supraglottic cancer. Otolaryngol Head Neck Surg 1990;102:351-61.

13. Bocca E, Calearo C, De Vincentiis I, Marullo T, Motta G, Ottaviani A. Occult metastases in cancer of the larynx and their relationship to clinical and histological aspects of the primary tumor: a four-year multicentric research. Laryngoscope 1984;94:1086-90.

14. Weber PC, Johnson JT, Myers EN. The impact of bilateral neck dissection on pattern of recurrence and survival in supraglottic carcinoma. Arch Otolaryngol Head Neck Surg 1994;120:703-6.

15. Weber PC, Johnson JT, Myers EN. Impact of bilateral neck dissection on recovery following supraglottic laryngectomy. Arch Otolaryngol Head Neck Surg 1993;119:61-4.

16. Soto Varela A, Frade Fozales C, Rio Valerias M, Valez Reguerio M, Labella Caballero T. Efficacy of prophylactic elective neck dissection in the treatment of cancer of the larynx. Rev Laryngol Otol Rhinol (Bord) 1996;117:41-5.

17. Coates HL, Devine KD. Carcinoma of the supraglottic larynx: a review of 221 cases. Arch Otolaryngol 1976;102:686-9.

18. Suarez C, Lorente JL, Nunez F, Diaz C, Gomez J. Neck dissection with or without postoperative radiotherapy in supraglottic carcinomas. Otolaryngol Head Neck Surg 1993;109:3-9.

19. Gavilan C, Gavilan J. Five-year results of functional neck dissection for cancer of the larynx. Arch Otolaryngol Head Neck Surg 1989;115:1193-6.

20. American Joint Committee on Cancer. Manual for staging of cancer. Philadelphia, Pa: JB Lippincott, 1988:27-32.

21. Ghoury AF, Zamora RL, Sessions DG, Spitznagel EL, Harvey JE. Prediction of occult neck disease in laryngeal cancer by means of a logistic regression statistical model. Laryngoscope 1994;104:1280-4.

22. Schuller DE, Bier-Laning CM. Laryngeal carcinoma nodal metastases and their management. Otolaryngol Clin North Am 1997;30:167-77.

23. Ramadan HH, Allen GC. The influence of elective neck dissection on neck relapse in N0 supraglottic carcinoma. Am J Otolaryngol 1993;14:278-81.

24. Johnson JT. Carcinoma of larynx: selective approach to the management of cervical lymphatics. Ear Nose Throat J 1994;73:303-5.

25. Zeitels SM, Vaughan CW. Preepiglottic space invasion in "early" epiglottic cancer. Ann Otol Rhinol Laryngol 1991;100:789-92.

26. Dursun G, Keser R, Aktürk T, Akıner MN, Demireller A, Sak SD. The significance of preepiglottic space invasion in supraglottic laryngeal carcinomas. Eur Arch Otorhinolaryngol Suppl 1997;254(suppl 1):110-2.

27. Marks JE, Devineni VR, Harvey J, Sessions DG. The risk of contralateral lymphatic metastases for cancer of the larynx and pharynx. Am J Otolaryngol 1992;13:34-9.

28. Thawley SE, Ogura JH. Conservation laryngeal surgery and radical neck dissection. In: English GM, ed. Otolaryngology. Vol 5. Philadelphia, Pa: Harper and Row, 1985:1-52.

29. Cummings CW, Session DG, Weymuller EA. Atlas of laryngeal surgery. In: Trumbold C, ed. St Louis, Mo: CV Mosby, 1984:209-62.

30. Jackson BS, Rosenthall L, Attia E. Laryngeal lymphoscintigraphy. Laryngoscope 1981;91:2085-91.

31. Welsh LW, Welsh JJ, Rizzo TA Jr. Laryngeal spaces and lymphatics: current anatomic concepts. Ann Otol Rhinol Laryngol [Suppl] 1983;92(suppl 105):19-31.

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EXPERIMENTAL REINNERVATION OF A STRAP MUSCLE WITH A FEW ROOTS OF THE PHRENIC NERVE IN RABBITS

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In order to compare application of the roots of the phrenic nerve to the ansa hypoglossi for laryngeal muscle neurotization, 1 or more roots from the phrenic nerve were implanted into the right sternothyroid (RST) muscle of rabbits (n = 36). Controls were intact animals (in which RST innervation is provided by the ansa; n = 6) and denervated ones (n = 6). At 66 ± 2 days (mean ± SE) after neurotization, during quiet breathing, inspiratory electromyographic activity and isometric contraction force were observed in all reinnervated RST muscles (n = 24). During maximal inspiratory effort, electromyographic activity and force increased. In animals reinnervated by the C4 root alone, forces (46.22 ± 7.8 g) were significantly higher than in intact animals (10.83 ± 5.0 g). Retrograde labeling proved the phrenic origin of the neurotization. Electromyography of the diaphragm was recorded. We conclude that in rabbits, neurotization of a strap muscle by 1 or 2 roots of the phrenic nerve allows inspiratory contraction, even during quiet breathing. Such inspiratory activity is not observed in sternothyroid muscles of intact animals innervated by the ansa hypoglossi.

KEY WORDS — larynx, phrenic nerve, rabbit, reinnervation, strap muscle.

INTRODUCTION

Laryngeal reinnervation has been the subject of many studies during recent decades. Direct anastomosis of a severed recurrent nerve cannot achieve vocal fold motility, because of misdirected regeneration of axons to antagonist muscles. Progress has been made with the concept of selective reinnervation of abductor or adductor muscles.

The ansa hypoglossi[1-4] and phrenic nerves[5-13] have been successfully used for experimental reinnervation of the posterior cricoarytenoid (PCA) muscle. In spite of good results in animals, phrenicotomy cannot be routinely proposed in humans, because of its respiratory consequences. Is it possible to achieve neurotization of a muscle by a phrenic nerve transfer with limited diaphragmatic damage? This question was already addressed by Crumley[6,7] with the technique of the split phrenic nerve graft. However, this technique was not successful in humans.[14] Another solution could be the use of only 1 or a few roots of the phrenic nerve; this was performed with success in cats[10,12,13] and in dogs.[8]

In rabbits, we previously showed that the 3 phrenic nerve roots take independent routes before joining the main phrenic nerve.[15] We demonstrated that resection of the highest root or 2 roots of the right phrenic nerve can preserve valuable innervation and function of the remaining diaphragm.[16,17] However, can the preserved roots of the phrenic nerve be transposed in the neck, and achieve muscle neurotization more successfully than the ansa hypoglossi does? To answer these questions, we carried out neurotization of the sternothyroid (ST) muscle in rabbits with 1 or 2 roots of the right phrenic nerve and compared the activity of these muscles with that in intact animals (in which ST innervation is provided by the ansa).

MATERIALS AND METHODS

In New Zealand rabbits (Charles River, France) weighing 3,000 to 4,700 g, section of the roots of the right phrenic nerve and implantation of these roots in the homolateral denervated ST muscle were performed. The experiments were carried out in accordance with French laws concerning animals.

After premedication with diazepam, animals were anesthetized with ketamine hydrochloride (12.5 mg/kg) and chlorpromazine hydrochloride (0.625 mg/kg); the depth of anesthesia was adjusted to maintain abolition of the corneal reflex but retain spontaneous breathing. Each animal was placed in a supine position. By a medial cervicotomy, denervation of the right ST (RST) muscle was performed by resection of the ansa hypoglossi at the level of the carotid

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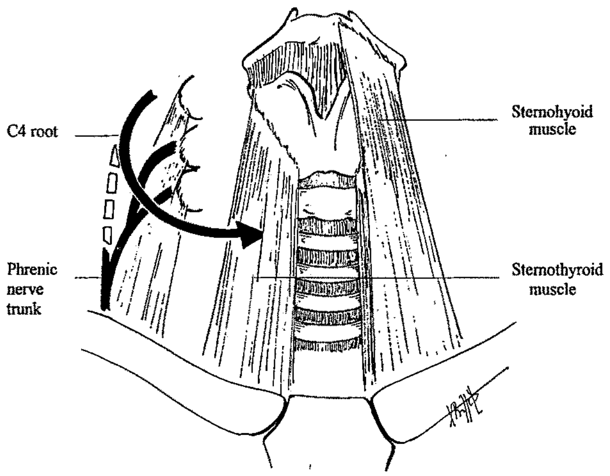

Fig 1. Implantation of C4 root of right phrenic nerve in right sternothyroid (RST) muscle.

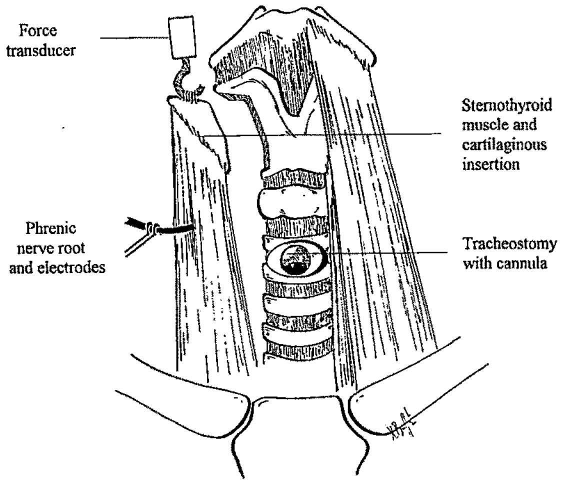
Force transducer
Phrenic nerve root and electrodes
Sternothyroid muscle and cartilaginous insertion
Tracheostomy with cannula

Fig 2. Attachment of right thyroid cartilage ala and RST muscle to isometric force transducer. Cannula was obstructed to induce inspiratory effort.

artery. The roots of the right phrenic nerve were exposed. Identification was controlled as previously described.[15]

The following procedures were performed (Fig 1). Group 3 (n = 12) had section of the right phrenic nerve, and implantation in the RST muscle. In group 4 (n = 12), the phrenic nerve root originating from C4 (C4 root) was sectioned and implanted in the RST muscle. In group 5 (n = 12), the common root originating from C5 and C6[15] was extended by a 2-cm-long nerve graft and then implanted in the RST muscle. The phrenic nerve root that originated from the anastomosis between the branches from C5 and C6 was too short to be transposed in the ipsilateral ST muscle. Therefore, it was extended by a free nerve graft removed from the superficial cervical plexus.

Implantation in the ST muscle was achieved by placement of the nerve between the ST muscle fibers, surrounded with biological glue (Biocol, LFB, Paris, France). A thin sheet of silicone was placed above the nerve graft to facilitate the discovery of the grafted nerve at the time of evaluation. The anastomosis between the branch from C5-C6 and the free nerve graft was carried out through a collagen cylinder (human IV/IV Ox collagen, Imedex, Chaponost, France) with a diameter of 1.5 mm, and 5 mm long, surrounded with biological glue. After disinfection, the wound was closed without drainage.

Two series of animals were used as controls. In group 1 (n = 6), denervation of strap muscles and a 2-cm-long resection of the right phrenic nerve were performed. In group 2 (n = 6), the intact animals, no surgical procedures were performed.

Two months after neurotization, the animals were anesthetized with the same technique. They were maintained in a supine position, their upper limbs attached to the surgical table, at a constant rectal temperature. A vertical cervicotomy was performed. The sternohyoid (SH) muscle — which is unpaired in rabbits — was divided at the midline. A tracheostomy was performed and the trachea was cannulated with a 4-mm–inside diameter endotracheal tube. In order to measure the intrathoracic pressure, for quantification of inspiratory effort, a 4.5-cm-long balloon catheter was placed transorally into the middle third of the esophagus and connected to a transducer (Statham PM 6, range ± 2.5 psi); the balloon was allowed to fill spontaneously at the end of an expiration.[18]

The phrenic nerve graft was then identified. The ST muscle was exposed and separated from the surrounding tissues except for its inferior third. The right thyroid cartilage ala was sectioned, carefully preserving the insertion of the ST muscle, and firmly attached to a force transducer (Metripak, 514 S/N OS, Gould, Longjumeau, France) placed in the initial position of the muscle (Fig 2). Signals were delivered to a WindoGraf recorder (Gould).

Through a laparotomy, the right and left hemidiaphragms were exposed. The electromyography (EMG) activity of the diaphragm was recorded during quiet breathing with a monopolar concentric needle electrode (Dantec, Type 13 L, Copenhagen, Denmark) connected to an EMG recorder (Racia, type 21 P, Le Bouscat, France). The following regions of the diaphragm were studied: sternal, anterior costal, midcostal, posterior costal, and crural regions on the right side; and sternal, midcostal, and crural regions on the left side. Tracings were kept for further analysis. A score was assigned by a double-blind scale from 0 to 3 according to the amount of EMG activity (0, no inspiratory activity; 1, limited inspiratory activity or neurogenic activity; 2, extensive inspiratory ac-

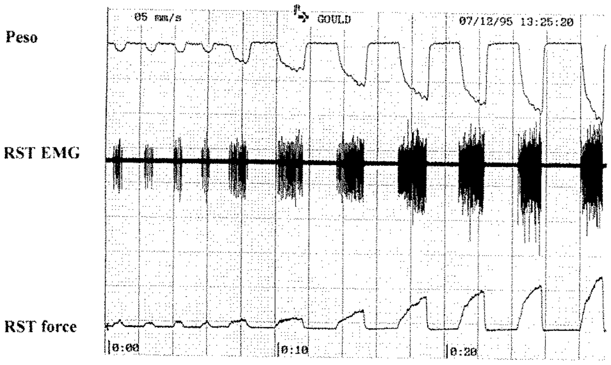

Fig 3. Simultaneous recording of esophageal pressure (Peso), electromyography (EMG) activity, and force of RST muscle before and after tracheal occlusion (increasing inspiratory effort, from left to right).

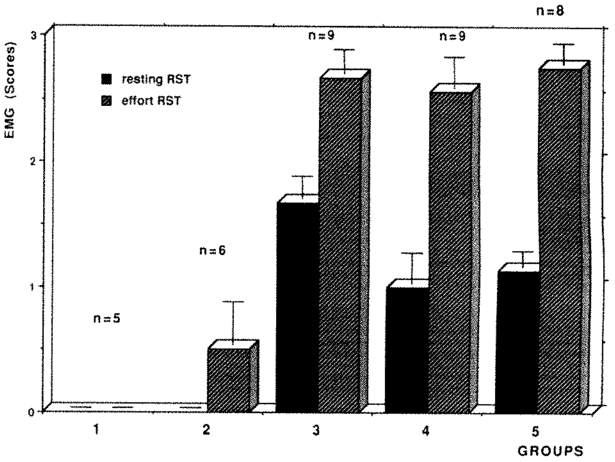

Fig 4. EMG scores of RST muscle during quiet breathing (resting RST) and during inspiratory effort (effort RST). Histograms are presented as mean ± SE. See comments in text.

tivity; and 3, inspiratory activity constituting an interference pattern).[16,19]

The EMG tracings from the RST and left ST (LST) muscles were recorded during quiet breathing and after prolonged occlusion of the trachea with the above-mentioned equipment. The recorded passband was between 65 Hz and 10 kHz. Tracings were kept for further analysis. The same scores as for diaphragmatic tracings were assigned by 2 scorers with a double-blind scaling. Animals that did not have inspiratory activity in the ST muscle during tracheal occlusion were considered failures and were not further evaluated.

The isometric contraction force of the RST and LST muscles was recorded during quiet breathing and after prolonged occlusion of the trachea (Fig 3). After these measurements, the grafted nerve was exposed and enclosed within a hooked wire electrode, and tetanic electrical stimulation was performed with 80 rectangular pulses per second of 20-mA intensity. Esophageal pressure (Peso) was continuously recorded on the WindoGraf recorder (Gould).

At the end of the experiment, Peso, ST EMG tracings, and isometric contraction force were recorded during the resection of the nerve of the ST muscle (phrenic or ansa) in order to confirm the disappearance of the signals after resection of the nerve.

In 2 animals of each group, retrograde staining of motoneurons was carried out with horseradish peroxidase (HRP). Forty-eight hours before EMG and force control, animals were operated on under general anesthesia. The RST muscle was exposed. Intramuscular injection of a 30% HRP solution (type VI, Sigma, Fluka Chemika Biochimika, St Quentin Fallavier, France) was performed with a microsyringe (Hamilton 701 RN, Bonaduz, Switzerland). Injections were stopped when the muscle was uniformly colored. A dose of 23 ± 2 μL was used. This staining was carried out under general anesthesia, through a cervicotomy, 48 hours before functional evaluation.

At the end of the evaluation procedure (EMG and force recording), a phosphate buffer saline solution was perfused into the heart of each animal. Then fixation was achieved with glutaraldehyde. The cervical spinal cord was removed and placed in a sucrose solution for 36 hours. The tissue was then frozen, and the spinal cord was sectioned in 30-mm slices. Staining was done as described by Mesulam et al.[20]

Statistical analysis was done with the nonparametric tests of Kruskal-Wallis and the Mann-Whitney U-test.

RESULTS

Three animals died. The surviving animals were evaluated 66 ± 7 days (mean ± SD) after the surgical procedure. The weights were 3,729 ± 463 g (range 3,000 to 4,700). Neither the elapsed time since surgery nor the weights within groups was significantly different.

In 8 animals of the reinnervated groups, no inspiratory EMG activity was found, even during tracheal occlusion (3 in group 3; 3 in group 4; 2 in group 5). These animals were considered failures of reinnervation; the evaluation procedure was not completed.

During quiet breathing, no EMG activity was observed in the RST muscle of denervated animals (group 1, n = 5) or healthy ones (group 2, n = 6). In successfully reinnervated animals (group 3, n = 9; group 4, n = 9; and group 5, n = 8), inspiratory EMG activity was observed during quiet breathing in all animals except for 2 of group 4. The mean EMG score of group 3 (1.63 ± 0.5; mean ± SD) was significantly higher than those of group 4 (1.00 ± 0.7) and group 5 (1.13 ± 0.4; $p < .05$; Fig 4).

During prolonged tracheal occlusion, no inspira-

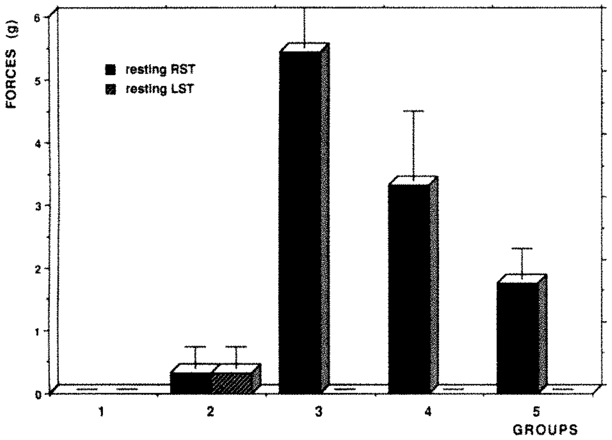

Fig 5. Isometric forces developed by RST muscle and left sternothyroid (LST) muscle during resting inspiration.

tory EMG activity was recorded in the RST muscle of the denervated animals (group 1); it was replaced by continuous fibrillation. The mean EMG scores of the RST muscle of the reinnervated groups (groups 3, 4, 5; respectively 2.63 ± 0.5, 2.56 ± 0.7, and 2.75 ± 0.4) were higher than those of group 1 (0.00) and group 2 (0.50 ± 0.8; $p < .001$). The EMG scores in group 4 (neurotization by the C4 phrenic root alone) were higher than those in group 2 (normally innervated by ansa hypoglossi). No significant difference was observed between the values of groups 3, 4, and 5 (Fig 4).

No EMG activity was recorded in the LST muscle during quiet breathing in any animal. During prolonged tracheal occlusion, light EMG activity appeared in all groups, no different from the right side in group 2 (healthy group).

The Peso (quantification of the inspiratory effort) was measured at the beginning of the EMG signal in the RST muscle. No RST EMG activity appeared in group 1. In group 2, EMG activity appeared for high inspiratory effort (at high values of Peso: 27.9 cm H_2O). In groups 3, 4, and 5, inspiratory RST EMG activity was present in quiet breathing (at low values of Peso: 2.6 to 5.7 cm H_2O). The difference was significant ($p < .01$) between group 2 and other groups, except for group 1. The differences between groups 3, 4, and 5 were not significant.

During quiet breathing, negligible force was developed in the RST muscles of healthy (group 2) and denervated animals (group 1). In reinnervated animals of groups 3, 4, and 5, an inspiratory isometric contraction force was observed. The force developed in group 3 (5.44 ± 2.55 g) was higher than those in group 5 (1.75 ± 1.4 g; $p < .01$) and group 4 (3.33 ± 3.4 g; not significant [NS]; Fig 5).

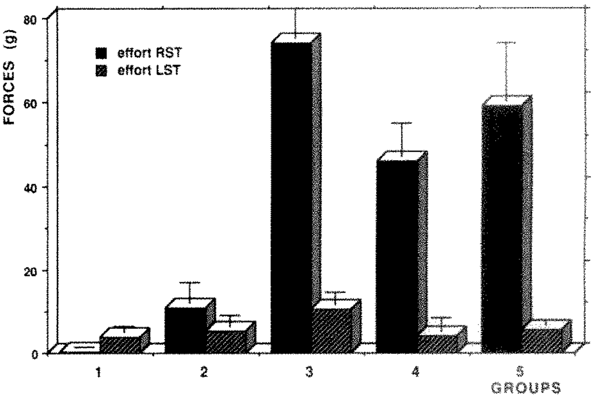

Fig 6. Isometric forces developed by RST and LST muscles during inspiratory effort.

During inspiratory effort, no inspiratory force was developed in the RST muscle of denervated animals (group 1). The mean force developed in the healthy unoperated group 2 (10.83 ± 12.4 g) was significantly lower than those in all the reinnervated groups ($p < .01$). A large variability of the individual values was observed. The mean value in group 3 (74.1 ± 25.3 g) was higher than those in group 4 (46.2 ± 23.0 g; $p < .05$) and group 5 (59.1 ± 39 g; NS; Fig 6).

In the LST muscle (innervated by the ansa hypoglossi), no force was recorded during quiet breathing in any animal. No significant difference appeared between values of the left and right sides of group 2 (healthy unoperated animals; Fig 5). During effort, a slight force was recorded in the LST muscle of the different groups, without a significant difference between groups (Fig 6).

Forces developed in the RST muscle during tetanic contraction (group 3, 114.3 ± 47.8 g; group 4, 63.6 ± 31.7 g; group 5, 90.1 ± 62.7 g) were compared to those obtained during tracheal occlusion. In animals reinnervated by roots of the phrenic nerve, a moderate but not significant difference was observed in

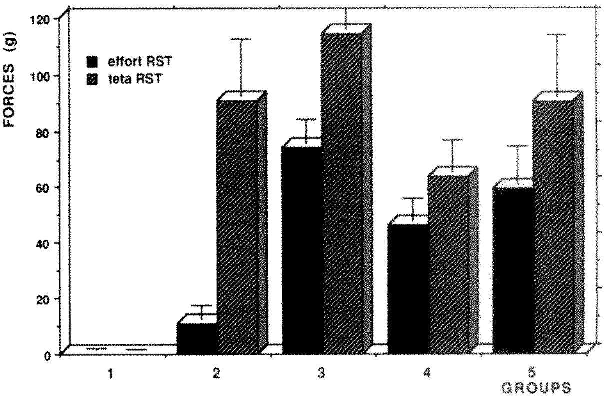

Fig 7. Isometric forces developed by RST muscle during inspiratory effort and tetanic contraction (supramaximal electrical stimulation of grafted nerve; teta RST). Stimulation was not performed in group 1 (non-reinnervated group).

METAMERIC LOCATION IN RIGHT CERVICAL SPINAL CORD OF CELLS STAINED BY HORSERADISH PEROXIDASE

Group	*Animal No.*	*C1-C2*	*C3-C4*	*C5-C6*
1	1	0	0	0
2	2	26	0	0
	3	45	0	0
3	4	0	10	6
	5	0	13	8
4	6	0	0	0
	7	0	4	0
5	8	0	2	11
	9	0	13	0

these two conditions (Fig 7). However, a large difference appeared in group 2 (innervation by the ansa hypoglossi): the tetanic contraction force (90.75 ± 49.1 g) was markedly higher than that seen during tracheal occlusion (10.83 ± 12.4 g; $p < 0.01$).

A correlation was observed between the forces developed in the RST muscle during tracheal occlusion and tetanic contraction in animals of groups 3, 4, and 5 ($r^2 = .697$; $p < .001$).

The values of tetanic contraction force of the LST muscle in different groups were not significantly different. There was no significant correlation between forces developed during dyspnea and those developed during tetanic stimulation.

In every case, resection of the grafted nerve was performed at the end of the experiment. The inspiratory EMG activity and the force developed by the ST muscle disappeared immediately.

The locations of stained cells in retrograde-labeled animals are represented in the Table. No stained cells were found in denervated animals. Motoneurons innervating the RST muscle fibers through the ansa hypoglossi were located in the first and second metameric cervical parts (C1 and C2) of the homolateral spinal cord (Fig 8).

Neurotization of the RST muscle was produced by motoneurons located at the homolateral C3 to C7 metameric levels, which are the locations of motoneurons of the phrenic nerve.[21] In group 4, in animal 6, no stained cells were found.

The EMG scores of the different portions of the diaphragm are summarized in Fig 9. In groups 1 and 3 (resection of the phrenic nerve but preservation of accessory phrenic nerve when present), residual EMG activity was recorded in the midcostal, posterior costal, and crural sections of the diaphragm.

No difference was observed between group 2 and group 4, except in the sternal portion of the right diaphragm. In the anterior costal portion, the scores of groups 4 and 5 were higher than that of group 3 ($p < .05$). In the crural portion, the score of group 4 was higher than that of group 3 ($p < .05$). The EMG activity was preserved in the lateral and posterior regions when the accessory phrenic nerve was preserved. Innervation was preserved in the whole diaphragm, except in the sternal portion, when only the highest root of the phrenic nerve was resected.

DISCUSSION

We showed in rabbits that it was possible to restore inspiratory contraction of a denervated strap muscle by its neurotization with just 1 or 2 roots of the right phrenic nerve. Inspiratory contraction was already present at rest and was maximal during ef-

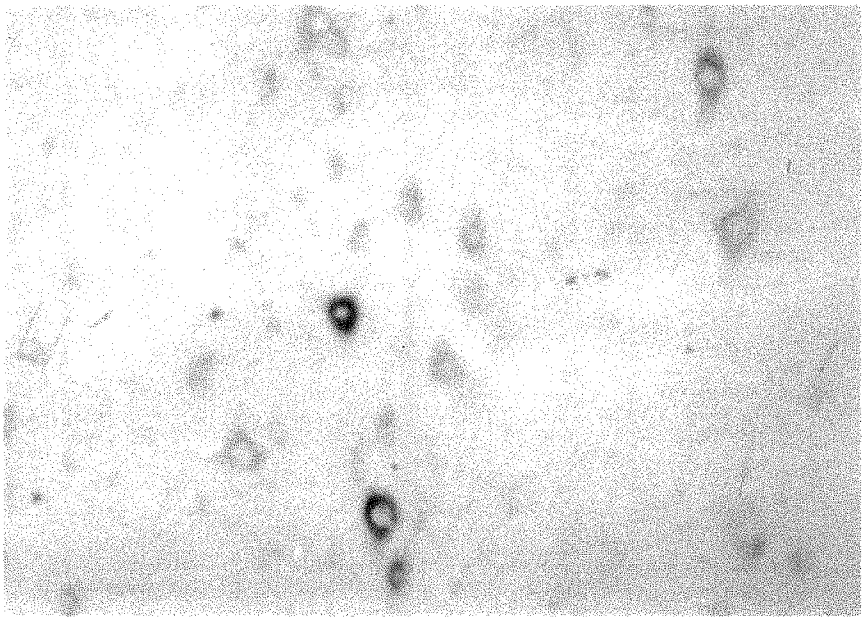

Fig 8. Cells stained by horseradish peroxidase located in right cervical spinal cord: cytoplasm appeared gray with punctuation induced by peroxidase. These marked cells are some of motoneurons of phrenic nerve.

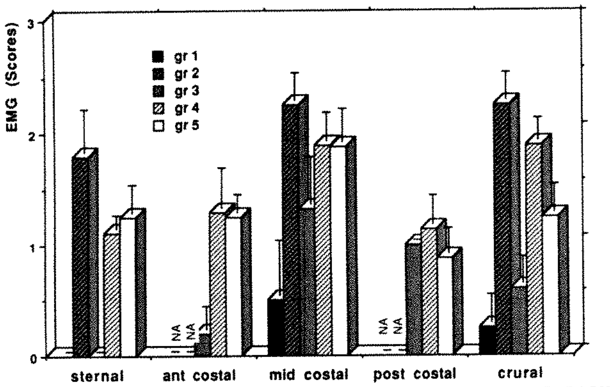

Fig 9. EMG scores of different portions of right diaphragm during quiet breathing (mean ± SE). EMG scores of anterior (ant) and posterior (post) costal portions were not studied in groups 1 and 2 (not applicable; NA).

fort. The intact ST muscle — innervated by the ansa hypoglossi — did not contract during quiet breathing; a light inspiratory contraction was observed during dyspnea, of less importance than in each reinnervated muscle, even with the highest root of the phrenic nerve. In these partially phrenicotomized rabbits, the right diaphragmatic innervation was preserved, particularly if only the C4 root was resected.

In this experiment, New Zealand rabbits were chosen because of previous studies performed in this animal involving neurotization of strap muscles[22-24] and phrenic nerve resection.[15-17] The neurotized muscles did not include the PCA muscles, because of the great difficulty in assessing the functional reinnervation of the larynx. On the contrary, a strap muscle was chosen, the ST, which was easy to access, either for neurotization or evaluation. The ST was preferred to the SH muscle, used by Broniatowski et al[23] and Meikle et al,[24] for the following reasons. The ST muscle was protected from fibrous scar by the SH muscle. Both ST muscles are anatomically separate, which is not the case for the SH muscle. In intact animals, the ST is innervated by a branch of the 12th nerve, which normally fires during deep inspiration (in dogs and humans[25,26]). In rabbits, the physiology of strap muscles is poorly known. If present, inspiratory contraction is observed in the ST muscle only during dyspnea (personal observations confirmed in the present study). In dogs, the nerve of the ST muscle has been used by different authors for the reinnervation of the PCA muscle.[3,4] Neurotization by direct implantation in the muscle was preferred to nerve anastomosis, because the diameter of the phrenic nerve is much greater than that of the ansa.

The different groups compared in this study were determined in regard to the anatomic arrangement of the phrenic nerve roots[15] and the functional consequences of their resection, bearing in mind possible surgical application. In previous investigation, we showed that diaphragmatic consequences of the resection of the C4 root (and, to a lesser degree, the root originating from C5 and C6) were limited.[16,17] The C4 root was most often able to reach the larynx or the high part of the trachea, because of its long individual route and its high origin. The root originating from C5 and C6 was very short and could not reach the ST muscle.[15] For this reason, in group 5, we used a 2-cm-long nerve graft that was anastomosed with the phrenic nerve root. The approximation of both nerves was achieved by enclosing them with a collagen tube or vein segment in order to avoid axonal escape. It would be reasonable to apply such an extension of the grafted nerve for neurotization of the PCA muscle.

The reinnervated animals were compared to healthy ones, in which ST muscles were innervated by a branch of the 12th nerve. Then we compared the phrenic nerve to the ansa hypoglossi through test and control groups. However, in a neurotized muscle, not as many motoneurons can be restored as function in a healthy one. Consequently, the difference observed was probably minimal in comparison to the real difference between the nerves.

Evaluation of the results was essentially improved by the measurement of isometric contraction force. It is a method currently used to evaluate the quality of restored motoneurons.[27] Nerves, motor end plates, and muscles could be quantitatively analyzed. Errors in the measurement could have been the consequence of the dissection of the muscles, the positioning of the force transducer, or the attachment between the muscles and the force transducer. These errors, if present, were the same for each animal and were not a bias for the comparison between groups. The HRP labeling of the phrenic motoneurons was an additional proof of the validity of reinnervation. In group 4, only 1 of the 2 injected animals showed HRP-labeled cells. The failure can be explained by poor reinnervation or miscounting of stained cells.

Neurotization was achieved in 26 animals of the 34 that survived the surgery and recovery. Failures in reinnervation were explained by release of implanted nerves, induced by tension or infection. The proportion of failures was the same in all groups, and did not increase in the group in which a graft was used. Nevertheless, this study did not aim to compare different techniques of neurotization, but rather, the inspiratory potential resulting from the use of different nerves for reinnervation.

Our findings indicate that neurotization of a muscle by 1 or several roots of the phrenic nerve allowed inspiratory contraction. These observations were in accordance with those of Baldissera et al,[10] Mahieu et al,[12] and van Lith-Bijl et al[13] on the cat larynx. They obtained a functional PCA neurotization with 1 or 2 roots of the phrenic nerve. We showed that the inspiratory contraction appeared during quiet breathing, in agreement with diaphragmatic contraction, and was restored with just 1 root of the phrenic nerve. This inspiratory contraction was never present at rest with the ansa hypoglossi.

From the present findings, it may be hypothesized that if the ansa is used for the neurotization of the PCA muscle, its firing would be present only if opening of the glottis did not improve, thus limiting the increase of PCA contraction. In the ST muscle neurotized with a phrenic nerve root, maximal inspiratory effort led to maximal contraction force on the same level as that produced by tetanic stimulation. On the other hand, in rabbits, we clearly demonstrated that with the ansa, the force achieved during maximal inspiratory effort was never as high as during tetanic contraction. If this finding carries over to humans, these are strong arguments against use of the ansa hypoglossi to reinnervate the PCA muscle.

The highest values of the forces developed by the ST muscle in animals reinnervated with the phrenic nerve were close to the highest developed in healthy ones, and even greater if the main phrenic nerve was used (nonsignificant). It could be explained by training of the neurotized muscle, contracting for each inspiration. It could also be supposed that the histochemical properties of the neurotized ST muscle were transformed by the phrenic nerve. The properties of a muscle are actually determined by the nerve in charge of its innervation.[28] Further, the diaphragm and the PCA muscle have similar histochemical compositions: they are both rich in type I and IIa fibers.[18,29] This similarity could be another argument for preferring the phrenic nerve to the ansa for laryngeal reinnervation.

Though markedly compensated, diaphragmatic denervation is the major ethical obstacle to the use of the phrenic nerve for human laryngeal reinnervation. Preservation of functional innervation of the diaphragm can be achieved by resection of just 1 or a few roots of the phrenic nerve.[17] The EMG scores recorded in the rabbit diaphragm in the present study confirmed the topography of the denervation (then innervation), depending on the resected roots, and were consistent with our previously recorded data.[16] In rabbits, C4 was in charge of the anterior part of the diaphragm; the accessory phrenic nerve, often present, was in charge of the lateral and posterior regions. In rabbits, there are some variations from one animal to another in the distribution of the innervation between diaphragm regions, with overlap.[16] These observations are in accordance with the studies of Rikard-Bell and Bystrzycka.[21] In cats,[30,31] dogs,[32,33] and humans,[34] an anterior-posterior distribution seems to be found. It can be hoped that resection of only 1 or 2 roots of the phrenic nerve would preserve functional innervation in the human diaphragm.

The experimental model presented here was easy to realize and useful for quantifying muscle neurotization by an automatically firing nerve such as the phrenic nerve. It could also be used for studying the effects of neurotropic medications and to analyze the transformation of the contractile properties of muscles. Further experiments must be performed on intralaryngeal (PCA) muscles of large animals.

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REFERENCES

1. Tucker HM, Harvey J, Ogura JH. Vocal cord remobilization in the canine larynx. Arch Otolaryngol 1970;92:530-3.

2. Rice DH. Laryngeal reinnervation. Laryngoscope 1982; 92:1049-59.

3. Attali JP, Traissac L, Gioux M. Réhabilitation de l'abduction cordale par anastomose nerveuse sélective chez le chien. Rev Laryngol Otol Rhinol (Bord) 1990;11:397-9.

4. Tixier C, Reyt E, Mezin P, et al. Reinnervations laryngées sélectives chez le chien par microsutures nerveuses des branches de division du nerf récurrent. Ann Otolaryngol 1992;109:23-31.

5. Iwamura S. Functioning remobilization of the paralyzed vocal cord in dogs. Arch Otolaryngol 1974;100:122-9.

6. Crumley RL. Experiments in laryngeal reinnervation. Laryngoscope 1982;92:1-27.

7. Crumley RL. Selective reinnervation of vocal cord adductors in unilateral vocal cord paralysis. Ann Otol Rhinol Laryngol 1984;93:351-6.

8. Brondbo K, Hall C, Teig E, Dahl HA. Functional results after experimental reinnervation of the posterior cricoarytenoid muscle in dogs. J Otolaryngol 1986;15:259-64.

9. Baldissera F, Cantaremma G, Marini G. Abduction of the paralyzed vocal cords in the cat using selective laryngeal reinnervation by phrenic motoneurons. Laryngoscope 1986;96: 1399-404.

10. Baldissera F, Cantaremma G, Marini G, Ottaviani F, Tredici G. Recovery of inspiratory abduction of the paralyzed vocal cords after bilateral reinnervation of the cricoarytenoid muscles by one single branch of phrenic nerve. Laryngoscope 1989;99:1286-92.

11. Marie JP, Dehesdin D, Ducastelle T, Senant J. Selective reinnervation of the abductor and adductor muscles of the ca-

nine larynx after recurrent nerve paralysis. Ann Otol Rhinol Laryngol 1989;98:530-6.

12. Mahieu HF, van Lith-Bijl JT, Groenhout C, Tonnaer JAD, de Wilde P. Selective laryngeal abductor reinnervation in cats using phrenic nerve transfer and ORG 2766. Arch Otolaryngol Head Neck Surg 1993;119:772-6.

13. van Lith-Bijl JT, Stolk RJ, Tonnaer JA, Groenhout C, Konings PN, Mahieu HF. Selective laryngeal reinnervation with separate phrenic and ansa cervicalis nerve transfers. Arch Otolaryngol Head Neck Surg 1997;123:406-11.

14. Crumley RL. Phrenic nerve graft for bilateral vocal cord paralysis. Laryngoscope 1983;93:425-8.

15. Marie J-P, Lerosey Y, Dehesdin D, Tadié M, Andrieu-Guitrancourt J. Cervical anatomy of phrenic nerve roots in rabbits. Ann Otol Rhinol Laryngol 1999;108:516-21.

16. Marie JP, Laquerrière A, Lerosey Y, et al. Selective resection of the phrenic nerve roots in rabbits. Part I: Cartography of the residual innervation. Respir Physiol 1997;109:127-38.

17. Marie JP, Tardif C, Lerosey Y, et al. Selective resection of the phrenic nerve roots in rabbits. Part II: Respiratory effects. Respir Physiol 1997;109:139-48.

18. Ferguson GT, Irvin CG, Cherniack RM. Effect of corticosteroids on diaphragm function and biochemistry in the rabbit. Am Rev Respir Dis 1990;141:156-63.

19. Boyd WH, Basmajian JV. Electromyography of the diaphragm in rabbits. Am J Physiol 1963;204:943-8.

20. Mesulam MM, Hegarty E, Barbas H, et al. Additional factors influencing sensitivity in the tetramethyl benzidine method for horseradish peroxidase neurohistochemistry. J Histochem Cytochem 1980;28:1255-9.

21. Rikard-Bell GC, Bystrzycka EK. Localization of phrenic motor nucleus in the cat and rabbit studied with horseradish peroxidase. Brain Res 1980;194:479-83.

22. Neal GD, Duncan G, Sutton D, Cummings CW. Horseradish peroxidase studies in animals with neuromuscular transpositions. Ann Otol Rhinol Laryngol 1981;90:396-7.

23. Broniatowski M, Grundfest-Broniatowski S, Chou S, Nose Y, Tucker H. Correlation between histology and nerve excitability after reinnervation of paralyzed strap muscles in the rabbit. Otolaryngol Head Neck Surg 1990;103:889-96.

24. Meikle D, Trachy RE, Cummings CW. Reinnervation of skeletal muscle: a comparison of nerve implantation with neuromuscular pedicle transfer in an animal model. Ann Otol Rhinol Laryngol 1987;96:152-7.

25. Ellenbogen BG, Gerber TG, Conn RL. Accessory muscle activity and respiration. Otolaryngol Head Neck Surg 1981;89:370-5.

26. Fink BR, Bacek M, Epanchin V. The mechanism of opening of human larynx. Laryngoscope 1956;66:410-25.

27. Rice DH, Cooper SD. Contractile properties of canine thyroarytenoid muscle reinnervated from the ansa cervicalis. Ann Otol Rhinol Laryngol 1989;98:153-6.

28. Mira JC. Effects of repeated denervation on muscle reinnervation. Clin Plast Surg 1984;11:31-8.

29. Lacau Saint Guily J, Fardeau M. Muscles intrinsèques du larynx de l'homme. Caractéristiques histoenzymologiques des fibres musculaires. Ann Otolaryngol 1983;100:1-12.

30. Fournier M, Sieck GC. Somatotopy in the segmental innervation of the cat diaphragm. J Appl Physiol 1988;64:291-8.

31. Hammond CGM, Gordon DC, Fisher JT, Richmond JR. Motor unit territories supplied by primary branches of the phrenic nerve. J Appl Physiol 1989;66:61-71.

32. Landau BR, Akert K, Roberts TS. Studies on the innervation of the diaphragm. J Comp Neurol 1962;119:1-10.

33. De Troyer A, Sampson M, Sigrist S, Macklem PT. Action of costal and crural parts of the diaphragm on the rib cage in dog. J Appl Physiol 1982;53:30-9.

34. Hovelaque A. Anatomie des nerfs craniens et rachidiens et du système grand sympathique chez l'homme. Paris: Doin et al, 1927:363-85.

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IMAGING CASE STUDY OF THE MONTH

MENINGOENCEPHALIC HERNIATION INTO THE MIDDLE EAR

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INTRODUCTION

Meningoencephalic herniation into the middle ear is a rare but life-threatening condition. This report presents a case of a teenage boy with meningoencephalic herniation into the middle ear and discusses the radiographic appearance and the management strategy.

CASE REPORT

A 15-year-old boy was admitted to our department complaining of a 4-year history of hearing loss in the left ear. He had a history of being in a traffic accident at the age of 1 year, after which a left-sided temporal bone fracture was diagnosed. He had not experienced intracranial infection, cerebrospinal fluid (CSF) leakage, or epilepsy.

An otoscopic examination revealed a whitish, soft mass behind an intact tympanic membrane in the left ear (Fig 1). Neither pulsation of the mass nor effusion in the middle ear was observed. A pure tone audiogram revealed a moderate mixed hearing loss (pure tone average of 38 dB) with a slight increase in the bone conduction threshold in the left ear and normal hearing in the right ear. High-resolution computed tomography (HRCT) disclosed a bone defect measuring approximately 1.5 cm^2 at the tegmen of the aditus ad antrum and a soft tissue–density mass that occupied the antrum and the attic, extending down to the mesotympanum (Fig 2). No abnormalities were found in the ossicles. Magnetic resonance imaging (MRI) revealed that the mass was in continuity with cerebral tissue and isointense to the brain on T1- and T2-weighted images, without showing enhancement with gadolinium–diethylenetriamine pentaacetic acid administration. The dural line was interrupted at the tegmental defect (Fig 3). On the basis of these findings, meningoencephalic herniation was strongly suspected. Exploratory mastoidec-

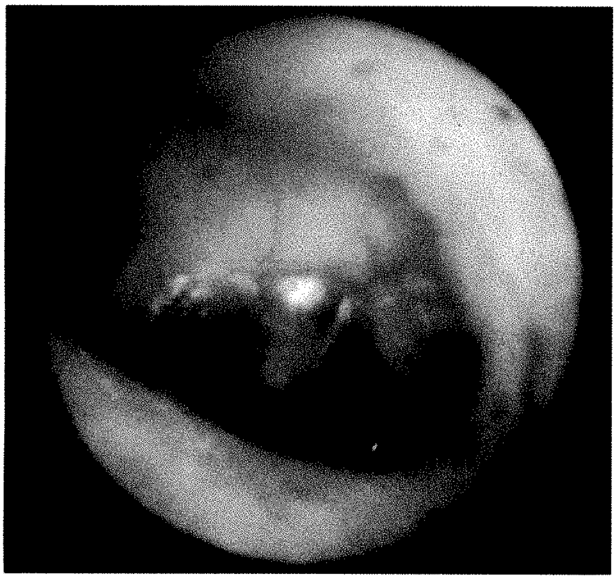

Fig 1. Whitish mass was visible behind intact tympanic membrane in left ear.

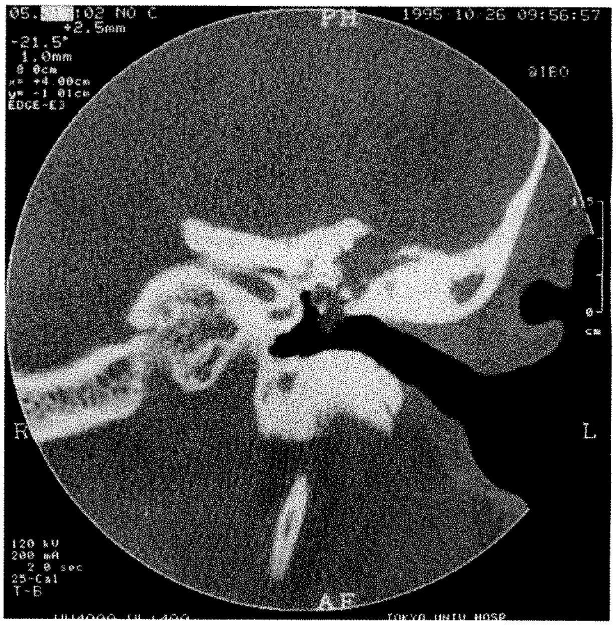

Fig 2. Nonenhanced high-resolution computed tomography reveals mass in attic and mesotympanum in continuity with tegmental bone defect.

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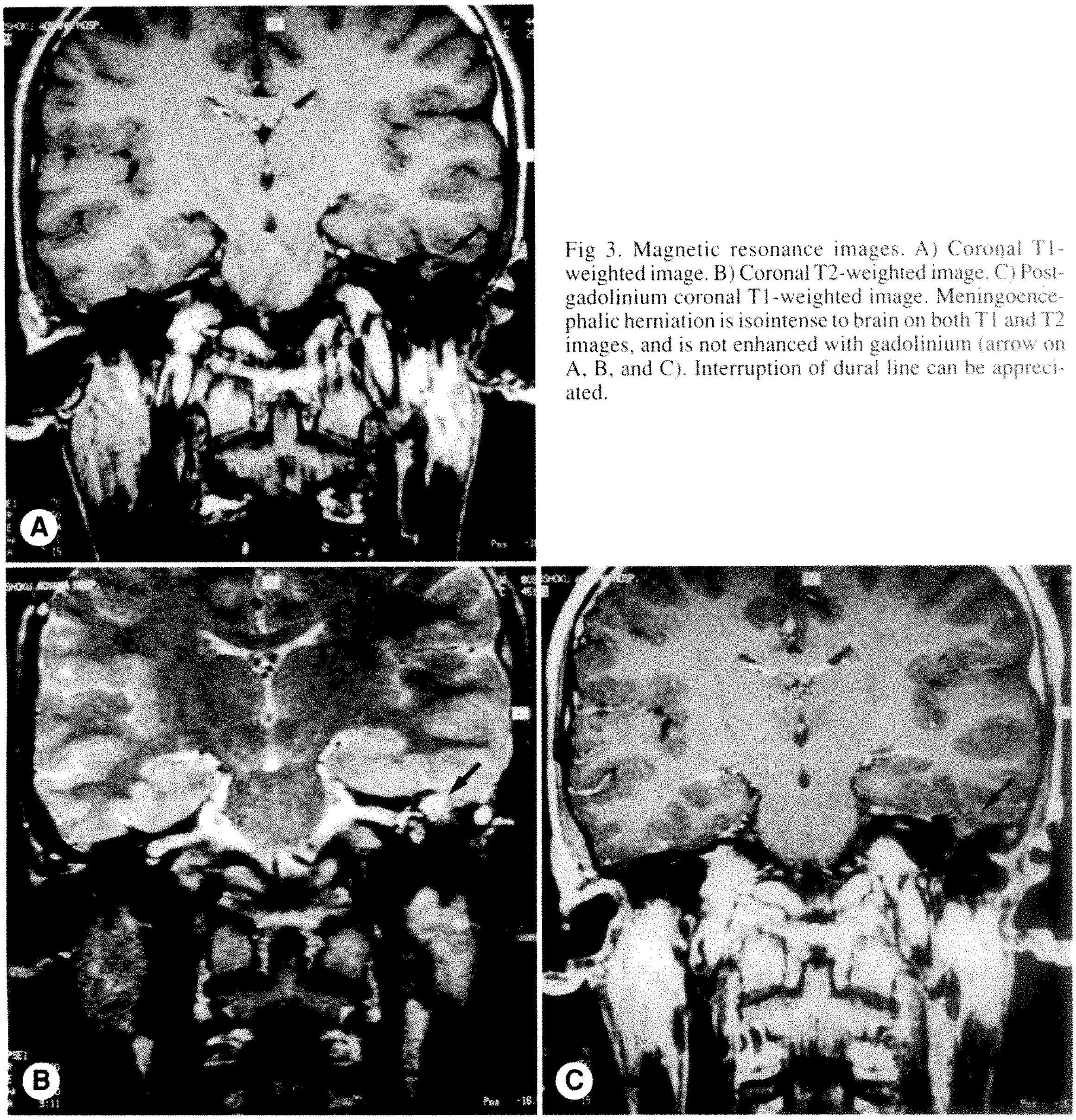


Fig 3. Magnetic resonance images. A) Coronal T1-weighted image. B) Coronal T2-weighted image. C) Post-gadolinium coronal T1-weighted image. Meningoencephalic herniation is isointense to brain on both T1 and T2 images, and is not enhanced with gadolinium (arrow on A, B, and C). Interruption of dural line can be appreciated.

tomy confirmed that the cerebral tissue was herniated into the middle ear space through the dural and bony defects of the tegmen of the aditus ad antrum. The herniated brain tissue appeared necrotic and was surrounded by granulation tissue. The tegmental defect was repaired with a periosteal free graft and a pedicled temporal muscle fascia flap by a combined middle cranial fossa approach. The herniated brain tissue was then totally removed from the middle ear space, with the ossicular chain left intact. The postoperative course was uneventful. The patient showed no signs or symptoms of intracranial infection, CSF leakage, or epilepsy. The bone conduction threshold showed no change, but the air conduction threshold slightly improved (by 5 dB on average).

DISCUSSION

Herniation of meningeal and/or encephalic tissue into the middle ear space is a rare condition that may occur in relation to infection, previous surgery, head trauma, or congenital defects.[1-21] Meningoencephalic herniation can occur at any age, but that associated with head trauma, which is regarded as a kind of growing skull fracture, is usually manifested in young adults as a sequela of accidents occurring in childhood.[1]

Highly suspicious clinical presentations of meningoencephalic herniation include intracranial infection,[1,9,12,17,18] CSF leakage,[1,3,4,7-9,11,14,16] epilepsy,[1,8,9,11,12,17,18] and aphasia.[1,8] The most common clinical picture, however, is conductive or mixed hearing loss or otitis media with effusion.[1,8,9,14,18-20] In the present case, mixed hearing loss was the only symptom. An increase in the bone conduction threshold was considered to have been caused by temporal bone fracture or contusion to the inner ear at the age of 1 year. A nonpulsating, whitish, soft mass was visible behind the intact tympanic membrane, mimicking congenital cholesteatoma.

A thorough radiologic assessment was very useful in establishing the diagnosis. On HRCT, herniated brain tissue is typically shown as a nonenhancing soft tissue–density mass in the attic and/or mastoid in continuity with a tegmental defect.[1] While HRCT can define the presence and size of the bony defect, it does not necessarily differentiate meningoencephalic herniation from other benign diseases such as cholesteatoma, granulation tissue, and cholesterol granuloma.[1,22,23] The ossicles are usually destroyed to some extent by chronic inflammatory disease, whereas they are typically intact in a case of meningoencephalic herniation. In the present case, HRCT showed no abnormalities of the ossicles, while the mass occupied the attic and antrum, with a large destruction of the tegmen. This finding allowed us to suspect the presence of meningoencephalic herniation rather than chronic inflammatory disease. Magnetic resonance imaging is beneficial to the differential diagnosis of these conditions. The herniated brain tissue is seen on MRI as a nonenhancing mass that is isointense to the brain in all sequences and is in continuity with the cerebral tissue.[1] Characteristically, an interruption of the dural line can be appreciated.[1,22,24] When these typical abnormalities are observed, the diagnosis of meningoencephalic herniation can be made before surgical exploration.

The management strategy of meningoencephalic herniation may differ depending upon the size and location of the tegmental defect and the presence of active infection. Small (<1 cm^2) or medium-sized (1 to 2 cm^2) defects can be managed through a transmastoid approach or sometimes with a minicraniotomy.[1] For large or anteriorly located defects or when active infection is present, a middle cranial fossa approach is recommended, because it principally avoids an additional transmastoid approach.[1] If necessary, a second surgery is performed to manage the middle ear condition. For the repair of the tegmental defect, most authors prefer to use elastic tissue (fascia, periosteum, or lyophilized dura) associated with a pedicled muscle flap and/or rigid tissue (cartilage or bone).[1-20] The use of heterologous material has been associated with a high rate of extrusion,[1,11,12,16] and the use of fascia alone has been associated with a high rate of recurrence.[1,11,12] Since this case had shown no symptoms of infection, the bone defect was medium-sized, and the site of the bone defect was relatively posteriorly located, the case could have been managed through a transmastoid approach, if necessary, with a minicraniotomy. We considered it necessary to remove the herniated brain tissue surrounding the ossicles for obtaining hearing improvement. Thus, after removal of the herniated tissue, we used a middle cranial approach to ensure the repair of the bone defect with a pedicled temporal muscle fascia flap. Few authors recommend reduction of the herniated tissue, provided that it is not infected and remains viable.[1,4,8,9,14,17,19] No residual dysfunction, however, has been reported after resection of herniated tissue.[1,7-11,13,14,16-18] This obviates the risk of leaving malfunctioning tissue that could be the origin of future dysfunction. Since the herniated brain tissue appeared necrotic and was thought to be the cause of hearing loss in the present case, it was totally resected. This resection did not cause any residual dysfunction. The postoperative air conduction threshold improved only slightly; perhaps the adhesion of the ossicular chain still remained.

REFERENCES

1. Aristegui M, Falcioni M, Saleh E, et al. Meningoencephalic herniation into the middle ear. A report of 27 cases. Laryngoscope 1995;105:513-8.
2. Hall C. Brain hernia: a postoperative complication in otology. Ann Otol Rhinol Laryngol 1939;48:291-310.
3. Stout JJ Jr, Trowbridge WV, Rugfles RL. Surgical repair of dural herniation into the mastoid bowl. Arch Otolaryngol 1969;89:850-5.
4. Fernandez-Blasini N, Longo R. Surgical correction of dural herniation into the mastoid cavity. Laryngoscope 1977; 87:1841-6.
5. Dedo HH, Sooy FA. Endaural brain hernia (encephalocele) diagnosis and treatment. Laryngoscope 1970;80:1090-9.
6. Bartels L, Lesley JL, Balis G, Bald C. Endaural brain hernia: repair using mastoid cortical bone. Am J Otol 1985;6 (suppl):121-5.
7. Paparella MM, Meyerhoff WL, Oliviera CA. Mastoiditis and brain hernia (mastoiditis cerebri). Laryngoscope 1978; 88:1097-106.
8. Glasscock ME, Dickins JR, Jackson GC, Wiet RJ, Feenstra L. Surgical management of brain tissue herniation into the middle ear and mastoid. Laryngoscope 1979;89:1743-54 .
9. Feenstra L, Sanna M, Zini C, Gamoletti R, Delogu P. Surgical treatment of brain herniation into the middle ear and mastoid. Am J Otol 1985;6:311-5.
10. Neely JG, Kuhn JR. Diagnosis and treatment of iatrogenic cerebrospinal fluid leak and brain herniation during or following mastoidectomy. Laryngoscope 1985;95:1299-300.
11. Bowes AK, Wiet RJ, Monsell EM, Hahn YS, O'Connor CA. Brain herniation and space-occupying lesions eroding the tegmen tympani. Laryngoscope 1987;97:1172-5.
12. Graham MD. Surgical management of dural and temporal lobe herniation into the radical mastoid cavity. Laryngoscope 1982;92:329-31.
13. Ramsden RT, Latif A, Lye RH, Dutton JE. Endaural cerebral hernia. J Laryngol Otol 1985;99:643-51.

14. Kemink JL, Graham MD, Kartush JM. Spontaneous encephalocele of the temporal bone. Arch Otolaryngol Head Neck Surg 1986;112:558-61.

15. Williams DC. Encephalocele of the middle ear. J Laryngol Otol 1986;100:471-3.

16. Kamerer DB, Caparosa RJ. Temporal bone encephalocele — diagnosis and treatment. Laryngoscope 1982;92:878-82.

17. Sterkers O, Attal F, Martin N, Julien N, Dessart I. Meningocerebral hernia and defects of the tegmen. Diagnosis and surgical treatment. Ann Otolaryngol Chir Cervicofac 1991;108:149-56.

18. Iurato S, Bux G, Colucci S, et al. Histopathology of spontaneous brain herniations into the middle ear. Acta Otolaryngol (Stockh) 1992;112:328-33.

19. Kaseff LG, Seidenwurum DJ, Nieberding PH, Nissen AJ, Remley KR, Pillon W. Magnetic resonance imaging of the brain herniation into the middle ear. Am J Otol 1992;13:74-7.

20. Wetmore SJ, Herrmann P, Fisch U. Spontaneous cerebrospinal fluid otorrhea. Am J Otol 1987;8:96-102.

21. Schurr PH. Endaural cerebral hernia. Br J Surg 1960;47:414-7.

22. Ahren C, Thulen CA. Lethal intracranial complications following inflation in the external auditory canal in treatment of serous otitis media and due to defects in the petrous bone. Acta Otolaryngol (Stockh) 1965;60:407-21.

23. Gavilan J, Trujillo M, Gavilan C. Spontaneous encephalocele of the middle ear. Arch Otolaryngol 1984;110:206-7.

24. Martin N, Sterkers O, Murat M, Hahum H. Brain herniation into the middle ear cavity: MR imaging. Neuroradiology 1989;31:184-6.

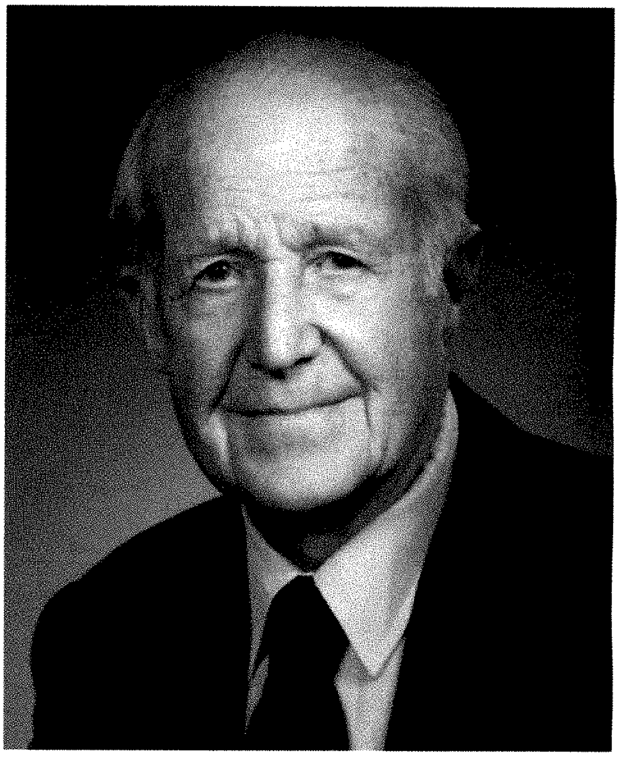
OBITUARY

GEORGE E. SHAMBAUGH, JR, 1903-1999

Everyone in otology agrees he was the greatest American otologist. In his 95 years he did it all: student, surgeon, teacher, scientist, author, innovator, husband, father, and role model to us all. From the preantibiotic otolaryngology of 1930, when he began his training at the Harvard Medical School–affiliated Massachusetts Eye and Ear Infirmary, through the introduction of the 1-stage fenestration operation by Lempert, to which he became one of the most important contributors, through the stapedectomy middle ear microsurgery era, to the acoustic neuroma, cochlear implant, skull-base surgery present era, he was always a leader, often the one out front.

His contributions to otology are too numerous to mention. First and foremost, he introduced to otology the operating microscope, which was slowly taken up by ophthalmologists, neurosurgeons, hand surgeons, and, ultimately, by all specialties of surgery. He saw the potential of seeing the operative site bigger and brighter, and persisted in the use of the microscope until everyone else came around, except Lempert, who said he saw "well enough" with a surgical loupe and never used the operating microscope, to his great disadvantage.

George Shambaugh, Jr, was born in Chicago, Illinois, on June 29, 1903. He was predestined to an otologic career as the son of George Shambaugh, Sr, a pioneer ear, nose, and throat specialist and one of the founders of the American Board of Otolaryngology. His high school education at University High School of the University of Chicago, and college training from Amherst College in 1924 prepared him for his medical education at Harvard Medical School, from which he graduated in 1928. An internship at Peter Bent Brigham Hospital, Boston, Massachusetts, from 1928 to 1930 preceded his resident training at the Massachusetts Eye and Ear Infirmary from 1930 to 1932 and at Presbyterian Hospital in Chicago from 1932 to 1933.



Beginning practice with his father in the Great Depression year of 1933, George had plenty of time to begin, under French Hansel, his study of allergy, which became a lifelong interest, and which he continued after he stopped operating at age 79. After hearing Julius Lempert present his 1-stage fenestration operation to the American Otological Society meeting in 1938, George became Lempert's first and most important student. In addition to being the first to use the operating microscope in otologic surgery, he introduced continuous irrigation of the lateral semicircular canal when creating the fenestra, both of which greatly improved on the original technique of Lempert. In Stockholm, Sweden, in 1938, George visited Leonard Holmgren, the first to make a fenestra in the lateral semicircular canal. While in Stockholm, George met Maurice Sourdille, who exteriorized the fenestra in a 3-stage fenestration operation. George created the first otologic research laboratory in the United States, at Northwestern Medical School, where he studied, in monkeys, the healing of the fenestra in the lateral semicircular canal, the cause of most failure in the fenestration operation. George reported on his animal research, and his experience with 800 consecutive fenestration operations in 1945, in a classic monograph published in the *Acta Otolaryngologica* in 1949, "Fenestration Operation for Otosclerosis." Lempert was so offended by George's additions to his original technique of fenestration, especially the use of the operating microscope, that he never spoke to George again, and an important association for otology was lost.

In 1946, George and Raymond Carhart, often referred to as the grandfather of audiology, developed the Otologic/Audiologic Diagnostic Clinic at Northwestern University Medical School. This unique weekly collaboration between otology and audiology was a marvelous training forum for masters, doctoral, and postdoctorate candidates in audiology and otolaryngology residents.

The first edition of *Surgery of the Ear* was published in 1959, followed by subsequent editions in 1967, 1980, and 1990, to become the most important textbook on the rapidly growing subspecialty of otology.

The first of the "Shambaugh International Workshops on Otomicrosurgery" began in 1959. The exciting and detailed presentations on tympanoplasty by Horst Wullstein and Fritz Zollner from Germany, as well as lectures by American otosurgeons on their new experiences in stapes surgery, stimulated a truly amazing discourse between workshop faculty and attendees. Subsequent workshops were held in 1963, 1967, and 1971, being combined in 1976 with the Shea Fluctuant Hearing Loss Symposium. Subsequent Shambaugh-Shea Workshops on Middle Ear Microsurgery were held in Chicago every 4 years, in 1980, 1984, and 1988, ending with the Ninth Shambaugh-Shea Workshop in 1992. These workshops had a truly international flavor, with participants from Europe, South America, Australia, and the Pacific Rim, as well as from all over North America.

Those individuals who attended the last, Ninth Workshop will remember that George Shambaugh missed most of the meeting due to an emergency operation, and against doctor's orders, he left his hospital bed to come to the final luncheon to speak to his cherished colleagues. Serendipity also provided a publishing forum for the workshop presentations through George's position as chief editor of the *Archives of Otolaryngology* of the American Medical Association from 1960 to 1970. In addition to being editor of the *Archives of Otolaryngology*, George published over 400 articles, right up to the time of his death.

Many awards were bestowed upon George in the form of presidencies of various professional societies and honorary awards by the world's most prestigious societies. Among his presidencies, those of the Otosclerosis Study Group, the American Society of Ophthalmologic and Otolaryngologic Allergy, and the American Otological Society reflected George's specialty interests in otology and allergy. However, his presidential leadership was also appreciated by more broad-based societies, such as the Triological Society and the Chicago Laryngological and Otological Society. Among other honorary degrees and awards, George received an honorary doctorate from the University of Freiburg in Germany in June 1967; the Award of Merit of the American Otological Society in May 1974; and an honorary doctorate from the University of Bordeaux in France in 1980.

When one contemplates the activities already mentioned, it is difficult to realize that in addition, George carried a heavy load in a private otologic group practice during the heyday of otosclerosis and mastoid and tympanoplasty surgery, and still found time for the chairmanship of the Department of Otolaryngology of the Northwestern University Medical School from 1951 to 1964. Reaching the status of professor emeritus in 1972 in no way altered his otologic office practice. His voluntarily stopping operating in 1982, at age 79, in no way reduced his office practice. It merely changed his medical orientation from otology and otologic surgery to otolaryngologic allergy, and did not alter his lifelong thirst for new ideas and new avenues of medical exploration.

George's marriage to Marietta Susan Moss, in 1927, resulted in the birth of 2 children: George III, born in 1931, and Susan, born in 1933. The marriage to Marietta (Mitzi) ended in 1945, and a second marriage, to Genevieve Krum in 1946, ended only with Gene's death in 1993. George and Gene's adopted son, David, provided them with great pride and joy as his career in the diplomatic corps and as a lecturer at the University of London progressed.

George Shambaugh, Jr, was a true American aristocrat of medicine: the son of a great otolaryngologist, the brother of 2 outstanding doctors, and a Harvard Medical School–educated and –trained academic, teacher, editor, and excellent private practitioner. He was certainly the greatest American otologist, who affected the lives of so many he taught directly or instructed through his writings, books, or deep personal relationships. George E. Shambaugh, Jr, was a true "Renaissance man," and we are all the better for having known him.

John J. Shea, Jr, MD
Eugene Derlacki, MD

Stuttering and Related Disorders of Fluency

Richard F. Curlee, editor. Second edition. Hard cover, illustrated, indexed, 307 pages, 1999. Thieme, New York, NY.

This is the second edition of a well-received text edited previously (1993) by Professor Curlee. Several of the 15 chapters are updates (Curlee's term) from the 1993 book; others are new. Some authors have published widely (Couture, Gregory and Gregory, Costello Ingham and Ingham); others, to my knowledge, have not.

A wide range of perspectives of stuttering and fluency is considered: early childhood, therapy for children, counseling, school-aged children and adolescents, adults, therapy for adults, and three chapters about "other kinds" of stuttering (so-called cluttering, acquired neurogenic disorders, and acquired psychogenic stuttering).

I found the final chapter to be especially noteworthy. It is interesting, informative, and somewhat unusual. It is unusual because the authors provide a succinct review, sometimes critical, of earlier chapters in the book. In my experience, rarely does that occur in a text like this. It is informative because Professor Siegel and Mr Gold make quick, easy-to-follow comparisons of the various approaches to stuttering. These comparisons are enlightening to someone like me and presumably others who are not stuttering specialists. The chapter is interesting because it is so well written.

To assist the reader of this review who has limited information about stuttering and fluency, I offer several summary statements about the disorders, identified primarily by Siegel and Gold in the final chapter.

1. There is apparently increasing support for the idea that stuttering is somehow physiological in nature.

2. There are a number of treatment programs that are reported to be successful.

3. However, the data about either the nature of stuttering or the effectiveness of treatment programs are yet insufficient or difficult to interpret.

4. Treatment programs are more effective for children than for adults. In any case, prompt referral to a speech-language pathologist for treatment is urgent.

5. The relationship between a given treatment program and theory (a conceptual model about stuttering) is frequently not well developed or at least not carefully identified.

Obviously, this is a significant text. The material in it offers the reader, albeit the sophisticated reader, an extensive discussion about the disorder of stuttering.

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An Introduction to Ear Disease

Bruce Black. Soft cover, illustrated, 86 pages, 1999. Singular Publishing Group, San Diego, Calif, $49.95.

Purpose: A comprehensive ear text at a basic level that represents a hands-on, ready-reference work for family physicians, medical students, junior otolaryngology residents, emergency room staff, nurses, and audiologists.

Contents: All aspects of the ear are covered, including otalgia. Clinical information is in line form, and there are pages of excellent tympanic membrane photographs depicting various pathologies and pictorial essays on middle ear prosthetics. The line drawings are also superb.

Attributes: This new textbook offers a modern and up-to-date guide to the practice of the ear for the needs of staff and referral personnel. Topics are approached in a problem-oriented manner with clinical information integrated throughout.

Limitations: When a book is not indexed, it takes time to find an entity. The section on vertigo needs a more organized approach.

DENNIS G. PAPPAS, MD
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Surgical Approaches in Otorhinolaryngology

Walter F. Thumfart, Werner Platzer, Andreas R. Gunkel, Herbert Maurer, and Erich Brenner. Hard cover, illustrated, indexed, 362 pages, 1999. Thieme, Stuttgart, Germany, $179.

Purpose: An atlas guideline to help surgeons select the correct approach and avoid errors. Illustrations are used to focus on typical or normal anatomic situations. Original drawings rather than anatomic photographs are utilized.

Contents: After delineating the typical head and neck divisions, approaches of local anesthesia and parotid surgery are described.

Attributes and Limitations: The volume clearly and beautifully describes surgical techniques to approaching standardized head and neck sites (disorders). Wordy didactic text is avoided and text portions are brief, and since the basic format is consistently the same for each approach, the book is easy to use. The authors have closely analyzed the steps of each procedure. Typically, the complex anatomic structures of head and neck surgery will require other sources of didactic information not included in this book. The drawings, for the most part, are realistic and faithful to true anatomy, though some are not.

Applicability: In considering the purchase of this book, one must consider that its main issue is not the lesion itself, but the surgical technique to approach the lesion. This beautiful book is of great value to head and neck practice for residents and senior surgeons dealing with the more major procedures (eg, transoral approach to the parapharyngeal space, rather than how to do a tonsillectomy).

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COMPLICATIONS OF PEDIATRIC LARYNGOTRACHEAL RECONSTRUCTION: PREVENTION STRATEGIES

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A retrospective chart review was performed to quantify the postoperative complications and outcomes of 82 consecutive cases of laryngotracheal reconstruction (LTR) and cricotracheal resection (CTR) performed at a pediatric tertiary care hospital over the last 9 years. Six cases of respiratory syncytial virus (RSV) bronchiolitis and 8 cases of cervical pseudomonal wound abscess (PWA) were identified in a total of 12 patients. All of these infections occurred after single-stage LTR or CTR. Both RSV bronchiolitis and PWA were associated with significantly more unexpected days of intubation and admission to the intensive care unit, as well as higher rates of failure of LTR. Ossification of the cricoid cartilage, grade IV subglottic stenosis, and untreated gastroesophageal reflux disease (GERD) were also associated with restenosis. Trisomy 21 did *not* significantly influence the success rate of pediatric LTR. Both RSV bronchiolitis and PWA are potentially preventable complications of pediatric LTR and CTR. We propose strategies to prevent these infections. We also advocate the treatment of GERD during the healing phase of LTR.

KEY WORDS — abscess, bronchiolitis, cricotracheal resection, gastroesophageal reflux disease, laryngotracheal reconstruction, *Pseudomonas*, respiratory syncytial virus, subglottic stenosis, trisomy 21, wound infection.

INTRODUCTION

Laryngotracheal reconstruction (LTR) and cricotracheal resection (CTR) are surgical procedures designed to correct stenosis of the subglottic larynx and/or the proximal trachea. The original two-stage laryngotracheal reconstruction (TSLTR) was introduced in 1972 by Fearon and Cotton.[1] Single-stage laryngotracheal reconstruction (SSLTR) was first reported in 1988 by Prescott.[2] Both types of LTR expand the airway lumen with a cartilage graft. With SSLTR, the tracheostomy is closed during the procedure and the reconstructed airway is stented with an endotracheal tube (ETT).

Cricotracheal resection was reported in children in 1993 by Monnier et al,[3] as an alternative to LTR in cases of severe subglottic stenosis (SGS). It involves circumferential resection of the distal cricoid and proximal trachea, followed by primary anastomosis. Single-stage laryngotracheal reconstruction and CTR are similar in that they are both single-stage procedures that require postoperative ETT stenting and management in a pediatric intensive care unit (ICU).

On the basis of the degree of stenosis and the procedure performed, the surgeon determines the optimal number of days that the child should remain intubated for stenting. Unfortunately, after SSLTR or CTR, certain postoperative complications may prevent timely extubation and potentially cause further morbidity. Several pediatric otolaryngologists have proposed strategies to prevent prolonged weakness from neuromuscular blockage.[4-6] Prevention of bacterial infections has also been addressed.[6] At our institution, significant morbidity from postoperative respiratory syncytial virus (RSV) bronchiolitis and pseudomonal wound abscess (PWA) prompted us to review our recent experience with the complications of LTR and CTR.

MATERIALS AND METHODS

From July 1989 to July 1998, 72 patients underwent 80 LTRs and 2 CTRs at The Children's Memorial Hospital in Chicago, Illinois. Charts were reviewed retrospectively to determine patient demographics and comorbidities, airway abnormalities, grade of laryngotracheal stenosis, type of reconstruction, and postoperative complications.

Preoperatively, all patients had a tracheostomy tube for at least 3 months. There were 60 single-stage procedures (58 SSLTRs and 2 CTRs). For each of these procedures, the expected number of days of intubation was noted. The number of *unexpected* days

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TABLE 1. DEMOGRAPHICS, COMORBIDITIES, AND AIRWAY ABNORMALITIES (N = 82)

	No.	*%*
Mean age (y)	3.6	
Male	48	59
Female	34	41
Comorbidities		
Reactive airway disease	44	54
History of prematurity	43	52
Chronic lung disease	37	45
Congenital heart disease	22	27
Immunodeficiency/steroid dependence	6	7
Trisomy 21	5	6
Other congenital syndromes	6	7
Malnutrition	2	2
Airway abnormalities		
Supraglottic/glottic		
Supraglottic collapse/laryngomalacia	8	10
Posterior glottic stenosis	8	10
Congenital anterior glottic web	2	2
Subglottic		
Subglottic stenosis (>50%)	75	91
Elliptical cricoid cartilage	7	9
Purely congenital subglottic stenosis	4	5
Ossified cricoid cartilage	3	4
Tracheobronchial		
Proximal tracheal stenosis	8	10
Trapped first tracheal arch	3	4
Fused tracheal arches	4	5
Complete tracheal rings	3	4
Primary tracheomalacia (>50%)	4	5
Primary bronchomalacia (>50%)	2	2

of intubation (UDIs) was recorded. The expected number of days in the ICU was considered to be 1 day more than the expected number of days of intubation. The number of *unexpected* days in the ICU (UDUs) was recorded for each single-stage procedure.

Infectious complications of SSLTR and CTR were grouped for statistical analysis. Group 1 consisted of 4 cases of isolated RSV bronchiolitis. Group 2 consisted of 6 cases of isolated PWA. Group 3 consisted of 2 cases of combined PWA and RSV bronchiolitis. Group 4 consisted of 3 cases of nonpseudomonal cervical wound abscess. One case of non-RSV bronchiolitis was excluded from statistical analysis. The control group consisted of the 44 cases of SSLTR or CTR that had none of these postoperative infections. The UDIs and the UDUs were the main outcome measures. The Wilcoxon 2-sample test was performed, comparing the UDIs and UDUs of each group to the UDIs and the UDUs of the controls.

Apparent risk factors for RSV bronchiolitis and PWA were analyzed. The diagnoses of RSV bronchiolitis, PWA, grade IV SGS, cricoid ossification, untreated gastroesophageal reflux disease (GERD), and trisomy 21 were also examined with regard to their effects on the outcomes of LTR and CTR.

RESULTS

Table 1 summarizes the demographics, comorbidities, and airway abnormalities of the 80 cases of LTR and 2 cases of CTR. Figure 1 summarizes the endoscopic grading of laryngotracheal stenosis and the reconstructive procedures performed. Table 2 summarizes the postoperative complications of TSLTR versus SSLTR and CTR.

Infections (Local and Regional). Six cases of RSV bronchiolitis and 8 cases of PWA occurred in a total of 12 patients. (Two patients had *both* RSV bronchiolitis and PWA.) Nonpseudomonal wound abscess occurred in 3 patients. Non-RSV bronchiolitis occurred in 1 patient. These infections only occurred in patients who underwent SSLTR (15) or CTR (1). Table 3 summarizes the statistical analysis of the UDIs and UDUs for patients with postoperative RSV bronchiolitis and PWA compared to patients without these infections. Statistical analysis revealed that these infections were associated with significantly more UDIs and/or UDUs. Further, 3 of the 6 patients who developed RSV bronchiolitis experienced 1 or more acute hypoxic events.

All cases of RSV bronchiolitis occurred between

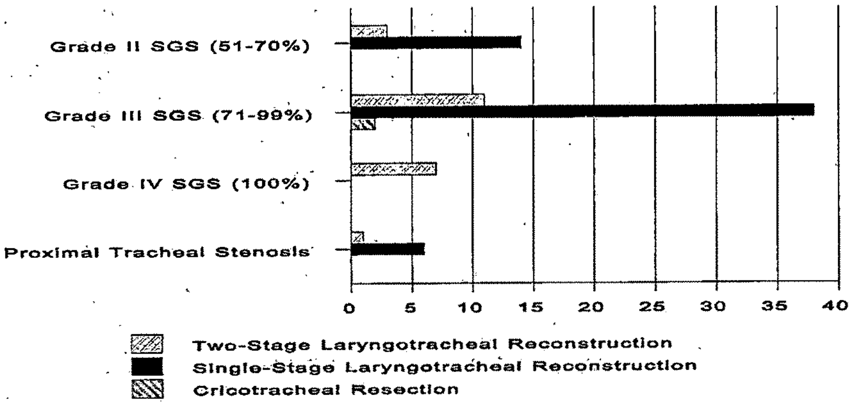

Fig 1. Grades of laryngotracheal stenosis found, and procedures used. SGS — subglottic stenosis.

TABLE 2. POSTOPERATIVE COMPLICATIONS

	TSLTR (n = 22)		*SSLTR/CTR (n = 60)*	
	No.	*%*	*No.*	*%*
Local				
Wound abscess	0		11	18
Subcutaneous emphysema	0		10	17
Transient vocal cord paralysis	0		2	3
Inability to decannulate	10	45	NA	
Tracheotomy required	NA		9	15
Restenosis managed endoscopically	4	18	12	20
Regional				
Viral bronchiolitis	0		7	12
Acute hypoxic event	0		10	17
Bacterial pneumonia	2	9	9	15
Asthma exacerbation	1	5	5	8
Pneumothorax (self-limited)	0		1	2
Systemic				
Bacteremia	0		7	12
Postparalytic weakness	NA		6	10
Narcotic and/or benzodiazepine withdrawal	NA		5	8
Arrhythmia	0		4	7
Death	0		0*	

TSLTR — two-stage laryngotracheal reconstruction, SSLTR — single-stage laryngotracheal reconstruction, CTR — cricotracheal reconstruction, NA — not applicable.

*One patient died of respiratory syncytial virus bronchiolitis 22 months after SSLTR.

TABLE 3. EFFECT OF RSV BRONCHIOLITIS AND WOUND ABSCESSES ON LENGTH OF INTUBATION AND ICU ADMISSION FOLLOWING SSLTR OR CTR

Group	*Unexpected Days of Intubation (mean ± SD)*	*Unexpected Days in ICU (mean ± SD)*
Control (no RSV or WA) (n = 44)	0.84 ± 1.58	1.50 ± 2.16
Isolated RSV (n = 4)	2.75 ± 1.71 p = .008 (SS)	3.25 ± 2.06 p = .066 (NS)
Isolated PWA (n = 6)	8.50 ± 7.04 p < .001 (SS)	12.83 ± 11.16 p < .001 (SS)
RSV and PWA (n = 2)	7.00 ± 0 p = .005 (SS)	9.5 ± 3.54 p = .017 (SS)
Nonpseudomonal WA (n = 3)	1.33 ± 1.53 p = .280 (NS)	5.33 ± 2.31 p = .014 (SS)

p values were calculated from Wilcoxon 2-sample test.

RSV — respiratory syncytial virus bronchiolitis, ICU — intensive care unit, SSLTR — single-stage laryngotracheal reconstruction, CTR — cricotracheal resection, WA — wound abscess, SS — statistically significant, NS — not statistically significant, PWA — pseudomonal wound abscess.

fall and spring, from October to April (Fig 2). Four patients developed significant complications from RSV bronchiolitis. Three of these patients had a history of severe prematurity (less than 30 weeks' gestation) and chronic lung disease (CLD; formerly known as bronchopulmonary dysplasia). The fourth had a history of viral myocarditis. In the 2 patients who developed postoperative RSV bronchiolitis without significant complications, no risk factors for severe RSV bronchiolitis were identified.

Analysis of the risk factors for PWA revealed that 1 patient was on chronic steroid therapy for CLD. This patient developed both PWA and RSV bronchiolitis. Another patient who developed PWA received a postoperative diagnosis of chronic malnutrition. In addition to PWA, this patient developed *Pseudomonas* bacteremia and eventually developed severe glottic and subglottic stenosis.

All patients were treated perioperatively with ampicillin or a first-generation cephalosporin. No preoperative tracheal cultures were performed. Of the 7 patients who had *Pseudomonas* cultured from their ETTs postoperatively, 6 developed a PWA. Twelve other patients received intravenous gentamicin intraoperatively for subacute bacterial endocarditis prophylaxis. None of these patients developed a PWA.

A subcutaneous air leak occurred in both patients who developed both PWA and RSV bronchiolitis. A subcutaneous air leak was also noted in 3 of the 6 patients with isolated PWA and in 1 of the 4 patients with isolated RSV bronchiolitis. Every wound abscess was treated by incision and drainage and systemic antibiotics: empiric, then culture-directed.

Regarding long-term outcome, 1 of the 2 patients who developed both PWA and RSV bronchiolitis failed CTR (requiring tracheostomy). Additionally,

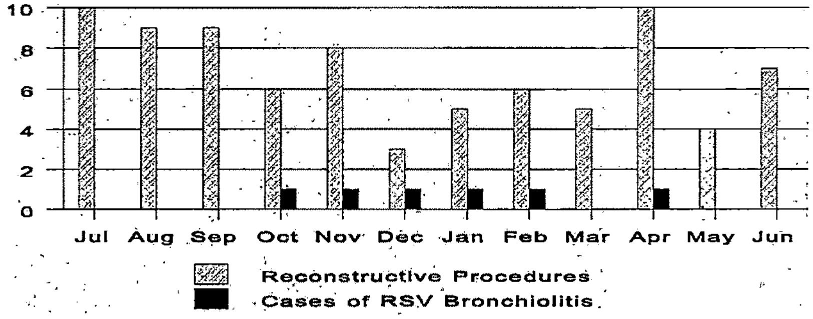

Fig 2. Seasonality of respiratory syncytial virus (RSV) bronchiolitis complicating laryngotracheal reconstruction.

1 of the 6 patients with isolated PWA failed SSLTR. This patient not only redeveloped SGS, but also developed a posterior glottic synechia and bilateral arytenoid fixation. Finally, 1 of the 4 patients with isolated RSV bronchiolitis also failed SSLTR. This patient had been born at 29 weeks' gestation with CLD and trisomy 21. Two months after SSLTR, tracheostomy was performed. Two years later, he again developed severe RSV bronchiolitis, and subsequently died of respiratory failure.

Single-stage laryngotracheal reconstruction was ultimately successful in the other patients with bronchiolitis or wound abscess. A variable number of postoperative endoscopic procedures were required to remove granulation tissue and dilate the subglottic larynx. In 1 patient with PWA, half of the auricular cartilage graft necrosed and collapsed into the airway. This portion of the graft was debrided endoscopically. Subsequently, the patient underwent 2 endoscopic carbon dioxide laser excisions of granulation tissue. Ultimately, the remaining cartilage graft epithelialized and the airway healed without stenosis. This patient required 11 days of ETT stenting, 13 days in the ICU, and 30 days of hospitalization. In contrast, another patient with PWA was treated by temporary tracheostomy, without endoscopic surgery. This patient was decannulated 6 months later.

Grade IV Subglottic Stenosis With or Without Ossified Cricoid Cartilage. Six patients had grade IV SGS. Intraoperatively, 2 of these patients were found to have complete ossification of the cricoid cartilage. For these 2 patients, a diamond bur was used to establish a subglottic lumen prior to anterior and posterior costal cartilage grafting and placement of an Aboulker stent. Neither patient could be decannulated after the initial TSLTR. In 1 patient, grade IV SGS redeveloped after both the first and a second TSLTR. The other patient was decannulated after a second procedure (SSLTR), but 2 years later required a single-stage tracheal resection to maintain an adequate airway.

Of the 4 patients with grade IV SGS and a nonossified cricoid cartilage, all were managed with a TSLTR. One patient pulled out her tracheostomy tube on postoperative day 5, tearing the wire through the Aboulker stent. Subsequently, the anterior costal cartilage graft necrosed. All of these patients had postoperative restenosis that prevented decannulation. One patient had a second TSLTR and was decannulated 6 months later.

Untreated GERD. In 4 patients, GERD was not diagnosed or treated until after an initial LTR failed. One of these patients initially had grade IV SGS with cricoid ossification. The other 3 patients, however, had no other identifiable risk factors for surgical failure. All 4 of these patients were treated for GERD for at least 1 month following a subsequent SSLTR or CTR; all 4 procedures were successful.

Trisomy 21. Five patients with trisomy 21 underwent LTR. As mentioned above, 1 patient with CLD developed postoperative RSV bronchiolitis, required tracheostomy, and eventually died of another bout of RSV bronchiolitis. Another patient required 4 UDIs because of cardiac arrhythmias. The SSLTR, however, was successful. The 3 other patients with trisomy 21 underwent LTR without significant complications.

DISCUSSION

RSV Bronchiolitis. Respiratory syncytial virus is considered the most important cause of viral lower respiratory tract infection (LRTI) in infants and small children.[7] Respiratory syncytial virus is an enveloped RNA virus of the paramyxovirus family. It infects and destroys the ciliated epithelial cells of the respiratory tract. A unique fusion protein enables this virus to form intraluminal aggregates (or syncytium) of the cells it infects.[8] These "syncytial cells" are pathognomonic of RSV infection (Fig 3). After RSV infection, mucociliary transport is compromised for weeks,[9] and the airway may remain hyperreactive for years.[10]

Transmission of RSV occurs by the droplet route. The RSV is spread by human hands and is inoculated through the nasal mucosa or the eyes. A droplet of nasal secretions with RSV remains infectious on skin or tissue paper for 30 minutes and on countertops for over 6 hours.[11] The RSV is not spread by long-distance small-particle aerosolization.[12] The incubation period of RSV is 2 to 8 days, with an average of 5 days. Viral shedding persists for 10 to 20 days.[13]

Respiratory syncytial virus has a worldwide distribution. In temperate climates, RSV causes annual cold weather epidemics, especially in urban areas. The peak months are during midwinter, but epidemics may begin as early as October or as late as May (in the northern hemisphere).[14] In tropical climates, RSV epidemics usually occur during the wet season.[15] In the southeastern United States, year-round RSV epidemics have been described.[16]

Infection with RSV is very common. At least half of all children are infected in their first year of life, and almost all children have been infected by their second birthday.[17] Reinfections by RSV occur throughout life and are most prevalent among preschoolers.

An RSV infection is heralded by clear rhinorrhea

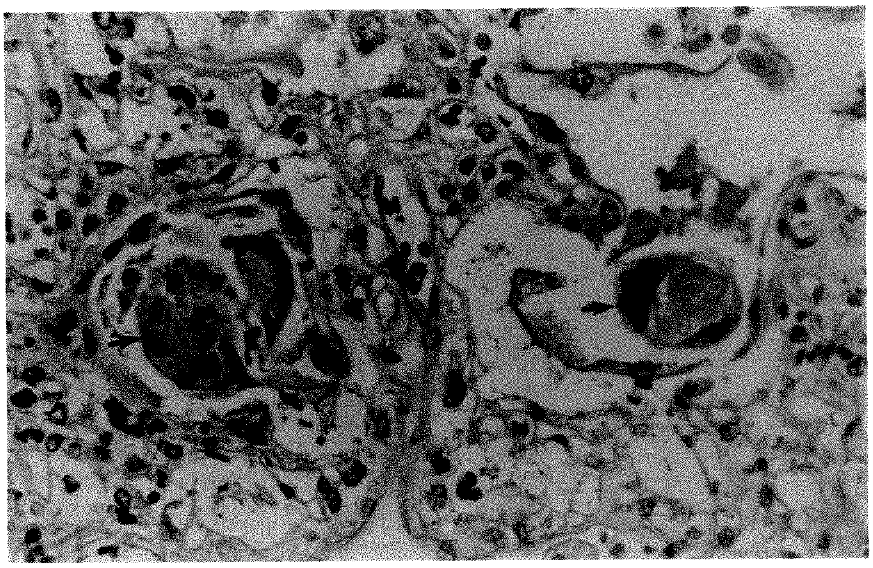

Fig 3. Syncytial cells (arrows) and inflammatory exudate obstructing small airways of child who died of severe RSV bronchiolitis (H & E, original ×200).

and fever. An LRTI occurs in about 40% of primary RSV infections.[18] In most children, an LRTI occurs by aspiration of infected nasal secretions. A tracheostomy or a nasotracheal tube, however, provides RSV with a direct conduit to the lower respiratory tract.

When RSV infects the lower respiratory tract, it usually causes bronchiolitis or pneumonia. Laryngotracheobronchitis (croup) accounts for less than 5% of RSV-induced LRTI. The severity of LRTI is quite variable. Approximately 2% to 3% of children with LRTI from RSV require hospitalization.[18]

Clinically, severe RSV bronchiolitis is characterized by fever, cough, tachypnea, retractions, diffuse wheezing, moist rales, hyperinflation, interstitial pneumonia, and atelectasis. Death from respiratory failure occurs in 1% to 5% of patients hospitalized for RSV.[8] Children who die of RSV usually have at least 1 of the major risk factors for severe bronchiolitis[7] (Table 4). In children who are otherwise healthy, reinfections by RSV are usually less severe than primary infections.[19] In children with risk factors for severe bronchiolitis, however, reinfection with RSV after the first year of life may still cause severe bronchiolitis and death.[7]

Our study revealed significant morbidity from RSV bronchiolitis complicating SSLTR or CTR. Morbidity was greatest in the children who had risk factors for severe RSV bronchiolitis. In fact, most children who undergo LTR or CTR have at least 1 risk factor for severe RSV bronchiolitis[6] (Table 4). Patients who undergo TSLTR may also develop RSV bronchiolitis. Children who undergo a single-stage procedure, however, tend to stay longer in the ICU and therefore are at greater risk for nosocomial transmission of RSV.[20]

TABLE 4. MAJOR RISK FACTORS FOR SEVERE RSV BRONCHIOLITIS

Prematurity
Chronic lung disease
Complex congenital heart disease
Pulmonary hypertension
Immunodeficiency
Cystic fibrosis

Information from Hall.[7]

The majority of RSV infections occurring after LTR could be avoided. Regardless of age, if the patient is at risk for severe RSV bronchiolitis, then LTR should be timed appropriately. The otolaryngologist should contact the local infectious disease specialist to determine the regional seasonality of RSV. In temperate climates, in which RSV infection consistently follows a seasonal pattern, LTR should *not* be performed on high-risk children during the RSV season. If an off-season RSV epidemic occurs in the ICU, then LTR should be postponed.

In regions in which RSV epidemics are unpredictable or year-round (eg, the southeastern United States), seasonal surgery may not be effective in decreasing the risk of postoperative RSV. For high-risk children in these regions, prophylaxis with intramuscular palivizumab or intravenous RSV immunoglobulin[21] may be considered prior to LTR or CTR. Consultation with the patient's pulmonologist would be prudent.

When any child undergoes LTR, all caregivers should observe standard infection control measures to prevent nosocomial transmission of RSV. Handwashing is of prime importance.[22] The patient should be isolated from other children for the entire hospital stay, starting in the outpatient surgery area, and ideally, should also be isolated from close contact with other children for at least 1 week preoperatively.

Pseudomonal Wound Abscess. One case of PWA complicating a TSLTR and causing subglottic restenosis was reported previously.[6] This is the first report of PWA complicating SSLTR or CTR. In our study, PWA caused significant immediate and long-term morbidity.

Pseudomonas aeruginosa is an aerobic gram-negative bacillus of the Enterobacteriaceae family.[23] It is a ubiquitous organism that grows in any moist environment. *Pseudomonas* preferentially binds to human tracheobronchial mucin.[24] In children who have an artificial airway for longer than 10 days, *Pseudomonas* is the most common respiratory pathogen.[25]

Destruction of grafted tissue is known to occur once the bacteria concentration reaches 10^5 bacteria per gram.[26] This bacterial concentration has also been shown to cause subglottic mucosal ulceration and subsequent chondritis of the cricoid following tracheostomy.[27] These experimental models support the hypothesis that PWA may have caused cartilage graft necrosis and subsequent recurrence of SGS in 2 of our patients.

In order to prevent pseudomonal and nonpseudomonal wound abscesses, we recommend that all patients who are scheduled for LTR or CTR have a preoperative tracheal aspirate for bacterial culture and sensitivity testing. This test should be performed between 3 to 7 days preoperatively, so that culture-directed intravenous antibiotics may be started at induction of anesthesia. If the culture and sensitivity testing reveal *P aeruginosa* that is sensitive to ceftazidime, and if the patient is not hypersensitive to cephalosporins, then ceftazidime would be the most appropriate antibiotic.[23] In order to avoid induction of resistant strains of *Pseudomonas*, the length of prophylactic antibiotic therapy should be limited to 48 hours or less. We therefore recommend discontinuing prophylactic antibiotics as soon as the drain site is closed and there is no sign of subcutaneous air leak, fluctuance, or cellulitis.

In addition to culture-directed antibiotics, several other measures may reduce the risk of wound abscesses. First, malnutrition should be corrected preoperatively and avoided postoperatively. Second, in order to prevent mechanical egress of contaminated tracheal secretions into the cervical soft tissue, an airtight seal of the cartilage graft should be obtained intraoperatively. This can be tested by flooding the wound with saline and ventilating to 30 cm H_2O pressure while observing for air bubbles. If an air leak cannot be eliminated with sutures, then a fibrin adhesive may be useful.[28] Postoperatively, the lowest peak pressures that provide adequate ventilation should be used. If the patient requires hand ventilation, then a manometer should be placed within the airway circuitry and observed during ventilation.

If a wound abscess develops despite these measures, then, in addition to incision and drainage and culture-directed antibiotics, we recommend a trial of endoscopic management. Debridement of necrotic tissue, carbon dioxide laser resection of granulation tissue, and reintubation may salvage the LTR. However, if the subglottis does not heal adequately or if the patient is unstable, tracheostomy may be necessary.

Combined RSV Bronchiolitis and Pseudomonal Wound Abscess. We postulate that the increased peak inspiratory pressures that were required to ventilate the patients with RSV bronchiolitis caused subcutaneous air leak and mechanical egress of *Pseudomonas* into the cervical soft tissues and thus promoted the formation of PWA.

Postparalysis Weakness and Narcotic and/or Benzodiazepine Withdrawal. Several recent studies have suggested specific measures to avoid postparalysis weakness and narcotic or benzodiazepine withdrawal after SSLTR. In 1995, a large series of patients managed successfully without neuromuscular blockage (NMB) was reported.[4] This management strategy was attempted in our patients who underwent SSLTR after 1995, but was successful in only a few, older children. In 1996, daily interruptions of NMB were recommended.[5] Again, this management strategy was adopted; however, our rate of postparalysis weakness and narcotic or benzodiazepine withdrawal did not change appreciably.

In a 1997 report, electrophysiological titration of NMB with daily recovery of neuromuscular function and avoidance of prolonged simultaneous NMB and corticosteroids was statistically proven to decrease weakness following extubation of children who underwent SSLTR.[6] This protocol merits further trials in children who are unable to tolerate nasotracheal intubation without pharmacological support. Clindamycin should be avoided in the setting of NMB, because it has been associated with delayed recovery of neuromuscular function.[29]

Grade IV SGS and Ossified Cricoid Cartilage. For patients with grade IV SGS, a 40% SSLTR decannulation rate was reported previously.[30] Our success rate with grade IV SGS was lower. Our study, however, had fewer patients, as well as 2 patients with ossification of the cricoid cartilage and 1 patient who traumatized her own cartilage graft. These problems have not been reported previously.

On the basis of our experience, we recommend consideration of cricotracheal resection for patients with grade IV SGS and severe grade III SGS, especially if the cricoid cartilage is ossified. To determine if the cricoid cartilage is ossified in a patient with a hard SGS, the laryngeal framework should be exposed and tested with a needle prior to harvesting of a cartilage graft. This would assist intraoperative decision-making without the risk of contaminating a potential graft site.

Untreated GERD. There is a high incidence of GERD in children with SGS.[6,31] Untreated GERD has also been associated with LTR failure.[32] Several experimental models have shown that when traumatized subglottic mucosa is exposed to hydrochloric acid and pepsin, greater inflammation and stenosis result.[33-35]

Our data, although retrospective and limited, support the hypothesis that untreated GERD contributes to the failure of LTR. Further, children with negative preoperative tests for GERD may still be at risk for postoperative GERD because of the presence of nasogastric and endotracheal tubes. Therefore, we recommend elevation of the head of the bed and pharmacological treatment of GERD in all patients who undergo LTR, at least until no raw mucosal surfaces or granulation tissue are visible.

Trisomy 21. In a previous report, 3 of 9 patients with trisomy 21 who underwent TSLTR required a second LTR to achieve decannulation.[36] In our study, 1 of 5 patients failed decannulation, with significant morbidity and eventual mortality caused by repeated RSV bronchiolitis. The LTR was successful in the other 4 patients with trisomy 21. We believe that LTR is appropriate in children with trisomy 21, with the caveat that the majority of these children have increased susceptibility to pulmonary infections, secondary to immunoglobulin G subclass deficiency.[37]

CONCLUSION

Significant complications of pediatric LTR and CTR include RSV bronchiolitis, PWA, and restenosis of the subglottic larynx. Based on our experience, the data reported herein, and our review of the literature, we propose the following prevention strategies.

1. Children at risk for severe RSV bronchiolitis should have either seasonal surgery or preoperative RSV prophylaxis, depending on the local RSV epidemiology.

2. All children should have a preoperative tracheal culture and receive culture-directed perioperative antibiotics. Intraoperatively, an airtight seal of the cartilage graft should be obtained.

3. Cricotracheal resection should be considered when the subglottic larynx is severely stenosed or ossified.

4. All children should be treated empirically for GERD until the subglottic larynx has healed.

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REFERENCES

1. Fearon B, Cotton R. Subglottic stenosis in infants and children: the clinical problem and experimental surgical technique. Can J Otolaryngol 1972;1:281-9.

2. Prescott CAJ. Protocol for management of the interposition cartilage graft laryngotracheoplasty. Ann Otol Rhinol Laryngol 1988;97:239-42.

3. Monnier P, Savary M, Chapuis G. Partial cricotracheal resection with primary tracheal anastomosis for subglottic stenosis in infants and children. Laryngoscope 1993;103:1273-85.

4. Rothschild MA, Cotcamp D, Cotton RT. Postoperative medical management in single-stage laryngotracheoplasty. Arch Otolaryngol Head Neck Surg 1995;121:1175-9.

5. Bauman NM, Oyos TL, Murray DJ, Kao SCS, Biavati MJ, Smith RJH. Postoperative care following single-stage laryngotracheoplasty. Ann Otol Rhinol Laryngol 1996;105:317-22.

6. Yellon RF, Parameswaran M, Brandom BW. Decreasing morbidity following laryngotracheal reconstruction in children. Int J Pediatr Otorhinolaryngol 1997;41:145-54.

7. Hall CB. Respiratory syncytial virus. In: Feigin RD, Cherry JD, eds. Textbook of pediatric infectious diseases. 3rd ed. Vol 2. Philadelphia, Pa: WB Saunders, 1992:1633-56.

8. Henderson FW. Viral respiratory infections. In: Rudolph AM, Hoffman JIE, Rudolph CD, eds. Rudolph's pediatrics. 19th ed. Norwalk, Conn: Appleton & Lange, 1991:651-3.

9. Aherne W, Bird T, Court SDM. Pathological changes in virus infections of the lower respiratory tract in children. J Clin Pathol 1970;23:7-18.

10. Webb MSC, Henry RL, Milner AD. Continuing respiratory problems three and a half years after acute viral bronchiolitis. Arch Dis Child 1985;60:1064-7.

11. Hall CB, Geiman JM, Douglas RG. Possible transmission by fomites of respiratory syncytial virus. J Infect Dis 1980; 141:98-102.

12. Hall CB, Douglas RG. Modes of transmission of respiratory syncytial virus. J Pediatr 1981;99:100-3.

13. Johnson KM, Chanock RM, Rifkind D. Respiratory syncytial virus: IV. Correlation of virus shedding, serologic response, and illness in adult volunteers. JAMA 1961;176:663-7.

14. Mufson MA, Levine HD, Wasil RE. Epidemiology of respiratory syncytial virus infection among infants and children in Chicago. Am J Epidemiol 1973;98:88-95.

15. Weber MW, Mulholland EK, Greenwood BM. Respira-

tory syncytial virus infection in tropical and developing countries. Trop Med Int Health 1998;3:268-80.

16. Halstead DC, Jenkins SG. Continuous non-seasonal epidemic of respiratory syncytial virus infection in the southeastern United States. South Med J 1998;91:433-6.

17. Brandt CD, Kim HW, Arrobio JO. Epidemiology of respiratory syncytial virus infection in Washington, DC. III. Composite analysis of eleven consecutive yearly epidemics. Am J Epidemiol 1973;98:355-64.

18. Parrott RH, Kim HW, Arrobio JO. Epidemiology of respiratory syncytial virus infection in Washington, DC. II. Infection and disease with respect to age, immunologic status, race, and sex. Am J Epidemiol 1973;98:289-300.

19. Glezen WP, Taber LH, Frank AL. Risk of primary infection and reinfection with respiratory syncytial virus. Am J Dis Child 1981;140:543-6.

20. Ditchburn RK, McQuillin J, Gardner PS. Respiratory syncytial virus in hospital cross-infection. Br Med J 1971;3:671-3.

21. American Academy of Pediatrics Committee on Infectious Diseases and Committee on Fetus and Newborn. Prevention of respiratory syncytial virus infections: indications for the use of palivizumab and update on the use of RSV-IGIV. Pediatrics 1998;102:1211-6.

22. Madge P, Paton JY, McColl JH, Mackie PK. Prospective controlled study of four infection control procedures to prevent nosocomial infection with respiratory syncytial virus. Lancet 1992;340:1079-83.

23. Fairbanks DNF. Pocket guide to antimicrobial therapy in otolaryngology–head and neck surgery. 9th ed. Alexandria, Va: American Academy of Otolaryngology–Head and Neck Surgery Foundation, 1999:46-7.

24. Vishwanath S, Ramphal R. Adherence of *Pseudomonas aeruginosa* to human tracheobronchial mucosa. Infect Immun 1984;45:197-202.

25. Brown OE, Manning SC. Microbial flora of the subglottis in intubated pediatric patients. Int J Pediatr Otorhinolaryngol 1996;35:97-105.

26. Liedburg NCF, Reiss E, Artz CP. The effects of bacteria on the take of split-thickness skin grafts in rabbits. Ann Surg 1955;142:92-6.

27. Sasaki CT, Horiuchi M, Koss N. Tracheotomy-related subglottic stenosis: bacteriologic pathogenesis. Laryngoscope 1979;89:857-65.

28. Kang DR, Leong H, Foss R, Martin P, Brooker CR, Seid AB. Sutureless cartilage graft laryngotracheal reconstruction using fibrin sealant. Arch Otolaryngol Head Neck Surg 1998; 124:665-70.

29. Brandom BW, Yellon RF, Lloyd ME. Recovery from doxacurium infusion administered to produce immobility for more than four days in pediatric patients in the intensive care unit. Anesth Analg 1997;84:307-14.

30. Cotton RT, Gray SD, Miller RP. Update of the Cincinnati experience in pediatric laryngotracheal reconstruction. Laryngoscope 1989;99:1111-6.

31. Walner DL, Stern Y, Gerber ME, Rudolph C, Baldwin CY, Cotton RT. Gastroesophageal reflux in patients with subglottic stenosis. Arch Otolaryngol Head Neck Surg 1998;124: 551-5.

32. Gray S, Miller R, Myer CM III, Cotton RT. Adjunctive measures for successful laryngotracheal reconstruction. Ann Otol Rhinol Laryngol 1987;96:509-13.

33. Gaynor EB. Gastroesophageal reflux as an etiological factor in laryngeal complications of intubation. Laryngoscope 1988;98:972-9.

34. Little FB, Koufman JA, Kohut RI, Marshall RB. Effect of gastric acid on the pathogenesis of subglottic stenosis. Ann Otol Rhinol Laryngol 1985;94:516-9.

35. Koufman JA. The otolaryngologic manifestations of gastroesophageal reflux disease (GERD): a clinical investigation of 225 patients using ambulatory 24-hour pH monitoring and an experimental investigation of the role of acid and pepsin in the development of laryngeal injury. Laryngoscope 1991;101 (suppl 53).

36. Miller RP, Gray SD, Cotton RT, Myer CM, Netterville J. Subglottic stenosis and Down syndrome. Am J Otolaryngol 1990;11:274-7.

37. Loh RK, Harth SC, Thong YH, Ferrante A. Immunoglobulin G subclass deficiency and predisposition to infection in Down's syndrome. Pediatr Infect Dis J 1990;9:547-51.

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PHARYNGEAL POUCH CARCINOMA: REAL OR IMAGINARY RISKS?

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Pharyngeal pouch or Zenker's diverticulum presents to the otolaryngologist with symptoms of dysphagia. As supported by the published literature, the condition is more frequently seen in Northern Europe, especially the United Kingdom, than elsewhere in the world. The cause of the reported increased incidence in the United Kingdom is not known, but may be dietary. Surgical management is the treatment of choice and is directed at the cricopharyngeus muscle by either an external or an internal approach. There is a real risk of carcinoma or carcinoma in situ developing in a treated or untreated pharyngeal pouch. Excision of the pouch sac is recommended in younger patients, less than 65 years, and in patients who have a large pouch. If endoscopic diverticulotomy is performed, then long-term patient symptom follow-up is to be advocated.

KEY WORDS — carcinoma, carcinoma in situ, pharyngeal pouch.

INTRODUCTION

Pharyngeal diverticula or pouches may be posterior, posterolateral, or lateral (pharyngocele), but the most commonly encountered type is the posterior pulsion diverticulum.[1] There is usually a single opening at the dehiscence of the muscles of the inferior constrictor (Killian's triangle); however, to date, 3 distinct varieties of double pharyngeal pouch have been described.[2] The disorder occurs more often in the northern part of Europe than in the southern part; it is common in the United States, Canada, and Australia, but rare in Japan and Indonesia.[3] A community study from the United Kingdom estimates an annual incidence of 2 per 100,000 people per year.[4]

Zenker's diverticulum is considered to be an acquired pouch located in the dorsal wall of the hypopharynx, immediately proximal to the transition from the hypopharynx to the esophagus; thus, it is also known as hypopharyngeal diverticulum or pharyngoesophageal diverticulum. The symptomatology is virtually pathognomonic: dysphagia, regurgitation of undigested food, weight loss, fetor ex ore, borborygmi in the cervical region, coughing, and repeated aspiration.[1] The duration of the symptoms can vary from weeks to years. Many different surgical methods have been described for the treatment of symptomatic pharyngeal diverticula. Currently, only 2 of them are applied with frequency: transcutaneous diverticulectomy and endoscopic treatment. It is difficult to quantify the incidence of hypopharyngeal diverticula in the general population, as not all cases are reported, and centers with a special interest in these diverticula attract cases from other areas, and thus have inflated figures in relation to population.[1]

The histology of the hypopharyngeal pouch, when excised, usually shows a sac containing an epithelial lining that is stratified squamous epithelium, and the submucosa often shows fibrous tissue surrounding it. Nearer the neck of the sac, scanty muscle fibers are found in the wall. Occasionally, and usually reported as case studies, there are variations of serious histopathologies found, in particular, squamous cell carcinoma and occasionally carcinoma in situ. Other histologic oddities have been reported, including ulceration of the pouch with underlying submucosal infiltration by plasma cells, lymphocytes, and eosinophils.[1] In a review[5] of squamous cell carcinoma reported occurring within a pharyngeal diverticulum, only 40 cases could be identified up to 1986, with another 3 cases reported since that time.[6-8] In cases with carcinoma, surgical excision was the only form of treatment to prolong life.[5] The main predisposing factor is thought to be chronic irritation and inflammation of the diverticular lining over many years, from food retention. A review[9] up to 1994 of carcinoma in situ occurring in a Zenker's diverticulum reports only 6 cases; all 6 patients were alive at time of reporting, tumor-free, after simple excision of the pouch.

Reviewed in this series are 91 cases of pharyngeal pouches (Zenker's diverticulum) diagnosed and

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treated at the Department of Otorhinolaryngology–Head and Neck Surgery at The University Hospital, Nottingham, England, over the period 1983 to 1997. We report 2 additional cases of pouch squamous cell carcinoma and 2 cases of carcinoma in situ. An updated review of the current literature is presented, and a suggested treatment algorithm is recommended in light of current surgical advances.

MATERIALS AND METHODS

During the period from 1983 to 1997, 91 patients presented to our department for diagnosis and treatment. Sixty of the 91 (65%) were 60 to 80 years of age, with 5 patients presenting under 50, and 3 patients over 90 years. All cases were investigated by a contrast radiology barium swallow study to confirm the clinical diagnosis. All pouches on diagnosis were classified by size, according to the Lahey[10] staging of I to III. Twenty patients had a stage I pouch, 47 had a stage II pouch, and 24 had a stage III pouch. Symptoms recorded at the time of presentation were dysphagia in 71 patients, regurgitation in 56, reflux symptoms in 39, globus sensation in 23, aspiration in 21, and weight loss in 18.

The recommended management of these patients was surgical in 78 and conservative (observation) in 13. The patients who elected to follow nonsurgical management included 2 patients who refused surgery after assessment, and the remaining 11 patients were followed up annually. All those who died did so of unrelated causes.

Surgical management was performed in 78 (64%) patients and included excision of the pouch and cricopharyngeal myotomy in 50 patients, Dohlman's procedure in 15, inversion of the pouch and cricopharyngeal myotomy in 7, and cricopharyngeal myotomy and dilation in 6.

All of the 50 pouches excised were sent routinely for histopathologic examination. The results identified 2 cases of carcinoma in situ and 2 cases of invasive squamous cell carcinoma. Only 1 case, that of a patient with a squamous cell carcinoma, was suspected and diagnosed prior to endoscopy. In the remaining 3 cases, the diagnosis was made only on histopathologic examination. This revealed an abnormal histopathology in 4 of 50 cases or an 8% risk of abnormality.

Of the 2 patients who had squamous cell carcinoma diagnosed, 1 was a 68-year-old woman who had undergone a Dohlman's procedure when 55 years of age. Because of unsatisfactory symptom improvement, she underwent a second Dohlman's procedure, 12 months later, with good symptom relief. After 10 years, her symptoms returned, and she underwent excision of the pouch. This surgery only gave a temporary relief of symptoms. The dysphagia had worsened after a period of some 12 months prior to her referral at age 66. Her barium swallow study prior to the most recent surgery did not reveal any abnormality. She remains well, asymptomatic and tumor-free, more than 6 years following the most recent excision of the pouch and cricopharyngeal myotomy.

The other patient was a 70-year-old man who had a squamous carcinoma in a pharyngeal pouch diagnosed prior to endoscopy. The barium showed a hold-up in the upper esophagus with an irregular filling defect in the pouch. At endoscopy, the tumor was extensive and staged T4N0. He underwent a pharyngolaryngectomy and gastric transposition. Unfortunately, he developed a pharyngogastrocutaneous fistula that required closure by a pectoralis myocutaneous flap. The patient died of bronchopneumonia 5 days after surgery.

The 2 patients who had carcinoma in situ identified in their excised pouch remain symptom-free and are free of tumor to date. One was a man of 76 years who had symptoms for 6 months prior to presentation. He had a type III pouch, underwent excision and myotomy, and remains well, tumor-free, at 3 years.

The second case was in a woman, 63 years old at presentation, who had symptoms for more than 5 years and had a type III pouch on barium examination. She was treated by excision of the pouch and cricopharyngeal myotomy and remains well and asymptomatic, more than 11 years following surgery.

In the latter 2 cases, endoscopy prior to excision did not show any abnormality in the pharyngeal pouch mucosa.

DISCUSSION

The first description of a pharyngeal pouch was made over 200 years ago, in 1769, by Ludlow of Bristol, who at postmortem discovered an abnormal dilatation of the posterior pharyngeal wall in a patient who had complained of dysphagia during life.[11] In 1877, Zenker and von Zeimssen,[12] in reviewing 5 cases of their own and 22 previous cases, clarified the then uncertain nature of a disease defined, as worded in Ludlow's title, as a "preternatural dilatation of a bag formed in the pharynx."[11]

A potential malignancy in a pharyngeal pouch should be suggested when there has been a sudden change in the severity of the normal symptoms, in particular, progressive dysphagia or aphagia. The patients often complain of more marked regurgitation

TABLE 1. CARCINOMA IN PHARYNGEAL POUCH FOLLOWING DOHLMAN PROCEDURE

Authors	*Year*	*Age When Cancer Diagnosed*	*Sex*	*Time From Surgery (y)*	*Treatment*	*Outcome*
Dohlman[13]	1949					No details given
Turner[14]	1963	63	M	30	Excision and radiotherapy	Alive and well with recurrence at 6 mo
Juby[15]	1969	66	M	5	Pharyngolaryngectomy	Neck recurrence at 12 mo
MacKay[16]	1976	54	M	11	Radiotherapy	Local recurrence (time unknown)
Stevens[6]	1989	91	M	2	Excision	Cardiac arrest at 2 d
This study	1999	65	F	5	Excision	No evidence of disease at 5 y

of sac contents, which sometimes may contain blood. Indeed, hematemesis may be the only presenting sign. Pain is an unusual symptom, but highly suggestive of malignant change, and has been described.[5] This type of clinical presentation occurred in both cancer patients presented. However, 1 of the patients had already been treated by Dohlman's procedure on 2 occasions, and the pouch had been excised previously, before worsening of her symptoms. Preoperative investigation did not reveal evidence of the seriousness of the disease within the pouch, which was only revealed on pathological examination. To date, she remains well, asymptomatic, and tumor-free. A review of the literature shows 5 previously reported cases of malignancy developing in a pharyngeal pouch that had previously been treated by Dohlman's procedure (Table 1[6,13-16]). Only 1 of the patients, who is reported here, had a long-term tumor-free survival. Surgery, if possible, would appear to be the preferred treatment in patients with anticipated long-term survival. However, the majority of patients treated by any means have died within 2 years.

In a thorough review of the published literature and reading of the original articles, the incidence of carcinoma in situ occurring in a pharyngeal pouch was 9 cases (Table 2[5,7,9,17-20]). The majority of cases (8/9 or 90%), at the time of reporting, were not diagnosed or suspected clinically or at the time of endoscopy, and all were diagnosed by histopathologic examination of the excised sac. All 8 patients had a large pouch presumed a stage III pouch and were in at least their sixth decade of life. The duration of symptoms, as recorded, did not give any clue to the results of the pathological examination, but all were "cured" of their high-risk neoplastic disease by simple surgical excision.

No treatment is indicated for a diverticulum with few symptoms, if the patient's general condition is poor, or for transitory diverticula. Clearly, each case must be judged on its individual merits, with the patient fully aware of the possible complications and potential benefits of operation. The basis of treatment is to correct the cause and remove the effect. It is agreed that pharyngeal pouches increase in size with time and that surgery is the preferred treatment. It is agreed by most surgeons that the cricopharyngeal sphincter is probably the main factor in the causation of posterior pharyngeal pulsion diverticulum, although the relationship between it and the pharyngeal constrictors in the causation is not yet understood.[3]

Radiologic examination of the upper alimentary tract provides a definitive diagnosis of a pharyngeal pouch. The radiographic study is incomplete if it does not include the lower esophagus, stomach, and duodenum, in order to look for other abnormalities.[1] The presence of an associated sliding esophageal hiatus hernia is a frequent finding and of itself may require treatment. The majority of pouches protrude to the

TABLE 2. CARCINOMA IN SITU IN PHARYNGEAL POUCH

Authors	*Year*	*Age*	*Sex*	*Duration of Symptoms*	*Size of Pouch*	*Treatment*	*Outcome*
Konjetzny[17]	1934	64	F			Excision	
Bullock and Snyder[18]	1952	66	M	3 y		Excision	NED at 5 mo
Zonnevylle et al[19]	1981	73	M	2 mo		Excision	NED at 12 mo
Baraka and Sadek[20]	1985	75	M	2 y		Excision	SCC at 5 y, radiotherapy, died
Bowdler and Stell[5]	1987	70	M	Months	No record	Excision	NED at 6 mo
Burton and Lund[7]	1990	83	M	3 y	Stage III	Excision	NED at 16 mo
Kerner et al[9]	1994	66	M	1 y	Stage III	Excision	NED at 36 mo
This study	1999	76	M	6 mo	Stage III	Excision	NED at 36 mo
	1999	63	F	5 y	Stage III	Excision	NED at 11 y

NED — no evidence of disease, SCC — squamous cell carcinoma.

left cervical region, while only 10% protrude to the right; this has suggested that the side the pouch occurs on might be determined by the handedness of the patient.[21] Barium swallow studies may demonstrate a filling defect, which needs to be differentiated from a food bolus. Food debris may move between films or repeated swallows. The diagnosis of carcinoma in a pouch is usually seen in the distal two thirds of the pouch, but can be easily missed, the diagnosis frequently being made at surgery, when careful examination with the esophagoscope should be performed. It is suggested that any loss of the smooth contour of the interior of a pouch seen on contrast radiographic studies should raise the suspicion of a tumor's being present. In a review of the literature, a radiographic diagnosis of carcinoma in a pharyngeal pouch was made preoperatively in 14 of 47 cases, suggesting an incidence of at least 29.8%.[5] All cases of carcinoma in situ had incidental findings on pathological examination of the diverticula.

There have been attempts to describe the size of the pouch as demonstrated by contrast radiology. One such system frequently referred to in published literature is by Lahey[10] (1930), who described 3 stages: stage I has small mucosal protrusions (the initial stage); stage II is a definite sac, but with the esophagus and hypopharynx still in line (the intermediate stage); and stage III is a large sac with the hypopharynx in line with the neck of the diverticulum and the esophagus inlet pushed anteriorly.

Other, more practical systems have been described that are easier to use and apply universally. The system used by Morton and Bartley[22] describes small pouches as less than 2 cm, medium pouches as 2 to 4 cm, and large pouches as more than 4 cm. Another method, used by van Overbeek and Groote,[3] uses cervical vertebra bodies as a standard size, describing the pouch as small if it is equal to or less than 1 vertebra in size; large pouches are more than 3 vertebrae in size.

Many different surgical techniques have been described for treating pharyngeal pouches. External approaches basically involve approaching the cricopharyngeal muscle band through the neck, identifying the pouch, and performing a cricopharyngeal myotomy. The pharyngeal pouch itself can be inverted,[22] mobilized, and suspended superiorly as a diverticulopexy,[23] excised, and closed traditionally by the suture technique, or can be excised and closed by a stapling device.[24] The endoscopic techniques essentially perform a diverticulotomy and an internal cricopharyngeal myotomy; the classic Dohlman's procedure divided the common septum with electrocautery.[25,26] Recent modifications of his method include the use of carbon dioxide[27] and potassium titanyl phosphate 532 lasers.[28] Currently, improved endoscopes and linear cutting stapling devices[29] have become very popular, with minimal hospitalization and return to normal swallowing within days of surgery.

Surgical treatment of a pharyngeal pouch is far from "routine surgery," with a morbidity ranging from 4% to 47% and a mortality rate of 0% to 5%.[30] An endoscopic approach is simpler and less time-consuming; however, the risk of major complications persists, with quoted rates of between 10% and 31%.[31] In considering treatment of a pharyngeal pouch in today's world of health care economics, cost-effectiveness of treatment and reduction in the time spent in surgery and follow-up is important. The advantage of an endoscopic method is a shorter anesthetic time combined with a reduced postoperative rehabilitation period. The recurrence rate, and patient satisfaction, frequently not alluded to, have been summarized by Parker and Hawthorne.[32] The recurrence rates vary considerably, from between 10% and 79% for Dohlman's procedure to between 2% and 33% for excision and myotomy in the UK series.[32] Short- and long-term follow-ups of patients testify to a satisfactory outcome in 90% or more patients treated by any surgical method.[4] One series states that almost 40% of patients treated had died, on average, at the age of 75.6 years, none of these deaths being related to surgery, and prolonged relief of symptoms had been achieved in all cases.[4]

Long-term assessment (>10 years) of swallowing after diverticulectomy with cricopharyngeal myotomy showed a multitude of abnormalities, even among those patients who were asymptomatic. Excision of the pouch does not appear to restore a normal swallowing mechanism.[33,34] In a comparison of endoscopic stapling and external pouch excision and cricopharyngeal myotomy, all patients treated by an external approach showed no residual pouch, whereas all patients who had endoscopic techniques performed showed some residual pouch.[35] However, it was remarked that all patients treated were "happy" with the outcome of their operation. A review[36] of current surgical practices of pharyngeal pouch surgery in England and Wales, carried out in 1995 and including otolaryngologists, general surgeons, and cardiothoracic surgeons, showed that pouch excision remained, at that time, the most common method in use. It was also remarked that the use of endoscopic stapling diverticulotomy was rapidly being adopted. In a recent series of publications on patient satisfaction, periods of hospitalization, and patients' return to "normal" swallow, surgeons concluded that endoscopic stapling should become the method of choice for the treatment of all pharyngeal pouches.[37-39]

All of the series that continue to support the option of conservative open surgical procedures — diverticulopexy, inversion or imbrication, and myotomy alone — or that advocate endoscopic methods such as the conventional Dohlman's coagulation method or endoscopic stapling, have not encountered a single case of pouch carcinoma or carcinoma in situ.[21-24,26-29,31-38,40] However, authors[4,30,39] who advocate endoscopic methods in general consider that large pouches (>4 cm) should be excised in the traditional fashion. The authors of this report agree with the algorithm suggested by Bonafede et al[30]: small pouches (stage I) can be treated by cricopharyngeal myotomy alone, but if the "sac" persists after myotomy, then an "inversion technique" is used if the persisting sac is small, including usually the smaller stage II cases; the remaining cases — large stage II and all stage III — should undergo a diverticulectomy and cricopharyngeal myotomy with histopathologic examination of the sac. Naturally, all patients who are to undergo surgical management of a symptomatic pouch must have endoscopic examination of the mucosal lining before any form of surgery. It must be noted that barium and endoscopy before surgery should diagnose the majority of cases of carcinoma. But cases of carcinoma in situ or small invasive carcinomas may be detected only at histopathologic examination of the pouch after excision. One current problem is for younger patients (60 years or less): should they undergo an endoscopic procedure (stapling or septum coagulation), there is likely to persist a radiologic pouch, in spite of relief of symptoms. Will the persisting pouch continue to be irritated by a sticking food bolus or other irritants? And, with the passage of time, will they be at risk of developing a carcinoma in situ and eventually invasive carcinoma?

It is to be currently recommended that all patients should be informed of the real but small risk that early carcinoma may be located in the pouch or develop at a later stage over the course of years, if the pouch is not excised at the initial presentation, and certainly if the pouch is stage II or stage III. It is currently important that patients who have been treated surgically have their posttreatment symptoms recorded and that the long-term natural history of conservative surgical procedures be documented. This should be done whether the patient's pharyngeal pouch has been managed endoscopically or by an external approach. It is only by long-term follow-up of such patients that the newer and seemingly "user-friendly" procedures can be introduced as standard procedure, and the "old-fashioned' methods abandoned.

To date, there has been documented 1 case of a man age 66 who refused surgical treatment 25 years previously, with a documented follow-up, who developed a small squamous carcinoma involving the diverticular wall, and he was successfully treated by surgery, radiotherapy, and concomitant chemotherapy.[8]

The natural history of a pharyngeal pouch is that it will get bigger with time, once muscle weakness in the inferior constrictor has commenced. The majority of patients present with symptoms in their seventh decade or older. Their symptom relief, with minimal morbidity, is the main goal of surgical treatment. However, the younger group of patients, those under the age of 65 years, should be considered for excision of the pouch with pathological examination and a long cricopharyngeal myotomy. Long-term follow-up should be considered. Endoscopic examination of the whole esophagus should be undertaken if symptoms persist or recur.

REFERENCES

1. Bowdler DA. Pharyngeal pouches. In: Kerr A, ed. Scott Brown's textbook of otorhinolaryngology. 5th ed. Oxford, England: Butterworth-Heinmann, 1997 (chapter 10, pages 1-21).

2. Meehan T, Henein RR. An unusual pharyngeal pouch. J Laryngol Otol 1992;106:1002-3.

3. van Overbeek JJM, Groote AD. Zenker's diverticulum. Curr Opin Otolaryngol Head Neck Surg 1994;2:55-8.

4. Laing MR, Murthy P, Ah-See KW, Cockburn JS. Surgery for pharyngeal pouch: audit of management with short- and long-term follow up. J R Coll Surg Edinb 1995:40:315-8.

5. Bowdler DA, Stell PM. Carcinoma arising in posterior pharyngeal pulsion diverticulum (Zenker's diverticulum). Br J Surg 1987;74:561-3.

6. Stevens DJ. Carcinoma of the pharyngeal pouch. J Laryngol Otol 1989;103:695-7.

7. Burton MJ, Lund WS. Pharyngeal pouch carcinoma: two unusual cases. J Laryngol Otol 1990;104:821-3.

8. Jorgensen T, Martinez Ramos C, Nunez Pena JR, Sanz Lopez R, Ruiz de Gopegui P, Tamames ES. Carcinoma in a long-standing Zenker's diverticulum. Rev Esp Enferm Dig 1994; 85:203-7.

9. Kerner MM, Bates ES, Hernandez F, Mickel RA. Carcinoma-in-situ occurring in a Zenker's diverticulum. Am J Otolaryngol 1994;15:223-6.

10. Lahey FH. The surgical management of pharyngo-oesophageal diverticulum. Surg Gynecol Obstet 1930;51:227-36.

11. Ludlow A. A case of obstructed deglutition from a preternatural dilatation of a bag formed in the pharynx. In: Medical observations and inquiries by The Society of Physicians London. Vol 3. 1769:85-101.

12. Zenker FA, von Zeimssen H. Krankheiten des Oesoph-

agus. In: von Zeimssen H, ed. Handbuch der speciellen Pathologie und Therapie. Vol 7 (suppl). Leipzig: FC Vogel, 1877:187.

13. Dohlman G. Endoscopic operations for hypopharyngeal diverticula. Proceedings of the Fourth International Congress of Otolaryngology (London). Vol 2. 1949:715-7.

14. Turner MJ. Carcinoma as a complication of pharyngeal pouch. Br J Radiol 1963;36:206-10.

15. Juby HB. The treatment of pharyngeal pouch. J Laryngol Otol 1969;83:1067-71.

16. MacKay IS. The treatment of pharyngeal pouch. J Laryngol Otol 1976;90:183-90.

17. Konjetzny GE. Krebsbildung in einem Pulsiondivertikel des Oesophagus und zur Chirurgischen Behandlung der Pulsiondivertikel. Zentralbl Chir 1934;61:8-12.

18. Bullock WK, Snyder EN Jr. Carcinoma in situ occurring in a pharyngeal diverticulum. Cancer 1952;5:737-9.

19. Zonnevylle JA, Waldeck KJJ, Hamming JJ. Carcinoma in situ in a pharyngo-esophageal diverticulum. Neth J Surg 1981; 33:94-7.

20. Baraka ME, Sadek SAA. Carcinomatous changes in pharyngeal diverticula. J Laryngol Otol 1985;99:297-9.

21. Stafford ND, Moore-Gillon V, McKelvie P. Handedness and the side on which pharyngeal pouches occur. Br Med J 1984; 288:815-6.

22. Morton RP, Bartley JRF. Inversion of Zenker's diverticulum: the preferred option. Head Neck 1993;15:252-6.

23. Watemberg S, Landau O, Avrahami R. Zenker's diverticulum: reappraisal. Am J Gastroenterol 1996;91:1494-8.

24. Westmore GA. The use of a stapling instrument in the surgery of hypopharyngeal diverticula. J Laryngol Otol 1983; 97:779-80.

25. Dohlman G, Mattsson O. The endoscopic operation for hypopharyngeal diverticula. Arch Otolaryngol 1960;71:744-52.

26. Van Overbeek JJM, Hoeksema PE. Endoscopic treatment of hypopharyngeal diverticulum: 211 cases. Laryngoscope 1982;92:88-91.

27. Holinger LD, Benjamin B. New endoscope for (laser) endoscopic diverticulotomy. Ann Otol Rhinol Laryngol 1987; 96:658-60.

28. Kuhn FA, Bent JP. Zenker's diverticulotomy using the KTP 532 laser. Laryngoscope 1992;102:946-50.

29. Koay CB, Bates GJ. Endoscopic stapling diverticulotomy for pharyngeal pouch. Clin Otolaryngol 1996;21:371-6.

30. Bonafede JP, Lavertu P, Wood BG, Eliachar I. Surgical outcome in 87 patients with Zenker's diverticulum. Laryngoscope 1997;107:720-5.

31. Bradwell RA, Bieger AK, Strachan DR, Homer JJ. Endoscopic laser myotomy in the treatment of pharyngeal diverticula. J Laryngol Otol 1997;111:627-30.

32. Parker AJ, Hawthorne MR. Endoscopic diverticulotomy versus external diverticulectomy in the treatment of pharyngeal pouch. J R Coll Surg Edinb 1988;33:61-4.

33. Zeitoun H, Widdowson D, Hammad Z, Osborne J. A video-fluoroscopic study of patients treated by diverticulectomy and cricopharyngeal myotomy. Clin Otolaryngol 1994;19:301-5.

34. Witterick IJ, Gullane PJ, Yeung E. Outcome analysis of Zenker's diverticulectomy and cricopharyngeal myotomy. Head Neck 1995;17:382-8.

35. Ong CC, Elton PG, Mitchell DM. Pharyngeal pouch endoscopic stapling — are post-operative barium swallow radiographs of any value? J Laryngol Otol 1999;113:233-6.

36. Koay CB, Sharp HR, Bates GJ. Current practice in pharyngeal pouch surgery in England and Wales. Ann R Coll Surg Engl 1997;79:190-4.

37. Scher RL, Richtsmeier WJ. Endoscopic staple-assisted esophagodiverticulostomy for Zenker's diverticulum. Laryngoscope 1996;106:951-6.

38. Koay CB, Bates GJ. Endoscopic stapling diverticulotomy for pharyngeal pouch. Clin Otolaryngol 1996;21:371-6.

39. Van Eeden S, Lloyd RV, Tranter RM. Comparison of the endoscopic technique with the more established procedures for pharyngeal pouch: results and patient satisfaction survey. J Laryngol Otol 1999;113:237-40.

40. Nguyen HC, Urquhart AC. Zenker's diverticulum. Laryngoscope 1997;107:1436-40.

IDENTIFICATION OF POSTERIOR CRICOARYTENOID MOTONEURONS IN THE RAT

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The posterior cricoarytenoid (PCA) muscle is the sole abductor of the larynx and is controlled by motoneurons located in the nucleus ambiguus. These motoneurons receive inputs from a variety of interneurons, including those that impart respiratory modulation, and are responsible for the phasic inspiratory activity of the PCA muscle. Identification of PCA motoneurons is therefore an essential initial step in understanding the mechanisms responsible for coordinated vocal cord abduction. We identified PCA motoneurons in the rat model by retrograde labeling, and following antidromic activation. A total of 194 neurons were identified by retrograde labeling with cholera toxin B subunit (CTB). Labeling was exclusively ipsilateral where the contralateral vagus and superior laryngeal nerves had been divided. The neurons were multipolar, with dimensions of 33.2 ± 6.4 μm (mean ± standard deviation) in length and 22.4 ± 3.4 μm in width. The neurons were located within a range of 0.6 to 2.4 mm caudal to the caudal pole of the facial nerve, 1.2 to 1.7 mm lateral to the midline, and 1.5 to 2.3 mm deep to the dorsal surface of the medulla. The PCA motoneurons were antidromically activated by focal stimulation of the PCA muscle. The extracellular field was recorded in 5 rats, and the PCA motoneurons were found within a range of 0.8 to 1.7 mm caudal to the caudal pole of the facial nerve, 1.5 to 2.0 mm lateral to the midline, and 1.9 to 2.4 mm deep to the dorsal surface of the medulla. The mean conduction velocity ranged from 37.0 ± 5.8 to 68.6 ± 5.0 m/s. An extracellular antidromic field potential, which corresponds to the distribution of the PCA motoneuron pool demonstrated by retrograde labeling with CTB, can be reliably obtained in a rat model following focal PCA muscle stimulation.

KEY WORDS — motoneuron, nucleus ambiguus, posterior cricoarytenoid muscle, retrograde labeling, vocal cord.

INTRODUCTION

Coordination of laryngeal muscle activity with respiration is essential to ensuring that vocal cord abduction occurs during inspiration and upper airway patency is maintained. The motoneurons responsible for abduction of the vocal cords are those controlling the posterior cricoarytenoid (PCA) muscle, which is the sole abductor of the larynx. Interneurons imparting respiratory control to these motoneurons are responsible for the characteristic phasic inspiratory activity of the PCA muscle, which has been well documented in both animals[1-12] and humans.[13-19]

The PCA motoneurons represent the final common pathway in motor control of the PCA muscle, and like other, similar motoneurons in the medulla and phrenic motoneurons in the spinal cord, they subserve a primary respiratory function. These cranial and spinal motoneurons display respiratory modulation of their activity and are regulated by a variety of interneurons in the ventrolateral medulla that are in turn controlled by other neurons in the brain stem. However, neither the circuitry nor the mechanism by which respiratory neurons interact to control the rate, depth, and pattern of breathing is clearly understood. The end product of these complex central integration and control systems is manifested by the phasic inspiratory activity of each PCA muscle fiber, as conveyed by the terminal divisions of the PCA branch of the recurrent laryngeal nerve.

The ability to maintain normal vocal cord abduction and upper airway patency is dependent on the integrity of the PCA motoneurons and their multiple neural connections. Study of the PCA motoneurons represents the first step in understanding the complex mechanisms responsible for coordinated vocal cord abduction and may provide an insight into the possible pathological processes underlying congenital bilateral vocal cord paralysis and other clinical manifestations of vocal cord dysfunction.

The PCA motoneurons are located in the nucleus ambiguus, which is a rostrocaudally elongated column of neurons embedded in the ventrolateral me-

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dulla. It contains visceral efferent neurons whose axons are distributed by the glossopharyngeal nerve, vagus nerve, and cranial root of the accessory nerve to supply the muscles of the palate, pharynx, larynx, and esophagus. Cardiac striated muscle and smooth muscle of the respiratory and gastrointestinal tracts are also supplied. The nucleus ambiguus is subdivided into a rostral esophagomotor compact formation, an intermediate pharyngomotor semicompact formation, a caudal laryngomotor loose formation, and a general visceromotor external formation.[20]

The orderly topographic distribution of laryngeal motoneurons in a rostrocaudal arrangement within the nucleus ambiguus of the cat and dog was first observed by Szentagothai[21] in 1943. He placed the cricothyroid motoneurons most rostrally, and then in caudalward order, the PCA, thyroarytenoid, lateral cricoarytenoid, and interarytenoid motoneurons. Lawn[22] in 1966 used retrograde degeneration techniques to observe regional localization of laryngeal motoneurons in rabbits. The cricothyroid motoneurons were in the rostral third of the nucleus ambiguus, and the motoneurons whose fibers were distributed by the recurrent laryngeal nerve were located in the caudal two thirds of the nuclear column.

Techniques currently used for identification of brain stem motoneurons include retrograde labeling and antidromic activation. The use of retrograde tracers for labeling motoneurons of individual laryngeal muscles was first described by Gacek,[23] in 1975, after he injected horseradish peroxidase into the individual laryngeal muscles of kittens, including the PCA, to trace retrograde axoplasmic flow into the cytoplasm of laryngeal motoneurons. The location of PCA motoneurons within the nucleus ambiguus in the ventrolateral medulla has also been demonstrated in a number of other species by means of horseradish peroxidase[24-33] and a variety of other retrograde tracers, including fluorescent dyes,[33-38] viruses,[39] and cholera toxin.[40]

Double-labeling techniques have also been successfully applied for simultaneous identification of PCA motoneurons and their connections. Holtman[38] demonstrated serotonin-containing and substance P–immunoreactive fibers and varicosities around retrogradely labeled PCA motoneurons, while Arita et al,[40] in a similar study, found serotonin-immunoreactive terminals surrounding the somata and proximal dendrites of PCA motoneurons.

Antidromic activation of a neuron can be elicited by stimulation of the peripheral part of the axon, which evokes an action potential that is conducted in a retrograde direction toward the cell body. This action potential then invades the cell body, from which it can be recorded extracellularly or intracellularly. Antidromic excitation, as described by Lipski,[41] was first used in extracellular studies, but could not be fully characterized until intracellular recording techniques were applied. The early extracellular studies were reviewed by Eccles,[42] while the initial intracellular studies were described in α-motoneurons by Brock et al[43] and Fuortes et al,[44] and in pyramidal tract cells by Phillips.[45]

Respiratory neurons of the ventrolateral medulla can be identified by their antidromic activation as being laryngeal or bulbospinal following stimulation of the vagus nerve and its branches or the spinal cord, respectively.

Both extracellular[46-51] and intracellular[52-56] recordings have been made from antidromically activated laryngeal motoneurons to specifically study their morphology and electrophysiological properties following stimulation of the cervical vagus, recurrent laryngeal, and superior laryngeal nerves. Motoneurons of an individual laryngeal muscle innervated by the recurrent laryngeal nerve, however, have not previously been identified by their antidromic activation, and current knowledge regarding PCA motoneuron location and morphology is limited to retrograde labeling techniques as outlined.

Identification of antidromically activated PCA motoneurons would require either stimulation of the PCA branch of the recurrent laryngeal nerve or focal stimulation of the PCA muscle itself. In a previous report from our laboratory, the intramuscular nerve distribution of the PCA branch of the recurrent laryngeal nerve in the rat was described.[57] The nerve and its superior and inferior terminal branches appeared to be too fine to isolate for direct stimulation; however, distribution of the nerve appeared to be confined to the PCA muscle. This finding suggests that focal stimulation of the PCA muscle would result in antidromic activation of PCA motoneurons alone. Further, the specific site of stimulation within the muscle does not appear to be critical, because despite the anatomic and functional evidence for compartmentalization, electromyographic studies have demonstrated an essentially uniform pattern of phasic inspiratory activity under general anesthetic throughout the PCA muscle.

A study was undertaken to identify PCA motoneurons in the rat by both retrograde labeling and antidromic activation.

Unconjugated cholera toxin B subunit (CTB) is a sensitive retrograde tracer when visualized by immunohistochemistry, especially in the central nervous system. In this study, PCA motoneurons were identified following application of CTB to the PCA muscle in rats both with and without prior division of the contralateral cervical vagus and superior laryngeal nerves.

The PCA motoneurons were antidromically activated by focal stimulation of the PCA muscle together with extracellular field recording, to map the distribution of PCA motoneurons within the ventrolateral medulla and compare the findings with those obtained by retrograde labeling with CTB.

METHODS

RETROGRADE LABELING

Animal Preparation. Experiments were conducted on 6 Sprague-Dawley rats (300 to 500 g) anesthetized with pentobarbital sodium (30 mg/kg) intraperitoneally. The larynx was exposed via a horizontal skin incision, and the inferior constrictor muscle was divided to allow rotation of the larynx to the left with exposure of the right PCA muscle. Care was taken to avoid damage to the right recurrent laryngeal nerve. The left cervical vagus and superior laryngeal nerves were divided in 4 of the 6 rats. We injected CTB (1%, List Biologicals, Campbell, Calif; 0.3 to 0.5 µL) using a micropipette into the right PCA muscle, and the neck incision was sutured. Those rats undergoing division of the left vagus and superior laryngeal nerves exhibited temporary stridor, but recovery was otherwise unremarkable. Rats were painlessly sacrificed 24 hours later — sufficient time to allow the retrograde axoplasmic transport of CTB into the brain stem.

Immunohistochemistry. Rats were perfused through the left ventricle of the heart with 200 mL of oxygenated tissue culture medium (Sigma Chemical Co, St Louis, Mo; D-8900), followed by 1 L of 4% formaldehyde in 0.1-mol/L phosphate buffer, pH 7.4. The brain stem was post-fixed overnight in the same fixative. Serial 50-µm transverse sections were cut through the brain stem and processed to reveal CTB immunoreactivity. Sections were incubated for 30 minutes in 10% normal horse serum (NHS) diluted with immunobuffer (IB; 10-mmol/L Tris, 0.9% sodium chloride, 0.05% thimerosal, Sigma Chemical Co, St Louis, Mo: T-5125, in 10-mmol/L sodium phosphate buffer containing 0.3% Triton X-100) and then in ExtrAvidin–horseradish peroxidase (1:500, Sigma Chemical Co) in IB containing 10% NHS for 24 hours. Sections were then incubated with goat antiserum to CTB (1:100,000, List Biologicals) in IB containing 10% NHS for 48 to 72 hours, followed by overnight incubations in biotinylated donkey anti-goat immunoglobulin (1:1,000, Jackson ImmunoResearch, Inc, West Grove, Pa) in IB containing 1% NHS, and a 4-hour incubation with ExtrAvidin–horseradish peroxidase (1:1,500, Sigma Chemical Co) in IB. The CTB immunoreactivity was revealed by an imidazole-intensified diaminobenzidine reaction (Llewellyn-Smith et al[58]). All incubations were carried out at room temperature with gentle agitation, and sections were washed in 3 changes of IB after each incubation. Sections were mounted onto glass slides, dehydrated, and cover-slipped in DePeX for analysis by light microscopy and color photography. Analog images were scanned (Kodak Slide Scanner) and saved as tagged image file format (TIFF) files. Small changes were made in brightness and contrast with Adobe Photoshop version 3.0.

ANTIDROMIC ACTIVATION

Animal Preparation. Extracellular field potentials were recorded from 5 adult Sprague Dawley rats (300 to 500 g). Anesthesia was induced by intraperitoneal injection of pentobarbital sodium (30 mg/kg) and maintained with additional intravenous doses (3 mg/kg) as required. Cannulae were inserted into the femoral vein and artery for drug administration and arterial blood pressure monitoring, respectively. Rectal temperature was monitored and a heating blanket and infrared lamp were used to maintain temperature at 37°C to 38°C.

By a dorsal paramedian approach, the scapula was retracted laterally to expose the brachial plexus and thereby allow identification of the roots of the phrenic nerve. A length of phrenic nerve was dissected and the nerve was divided distally and prepared for standard bipolar recording using silver electrodes. Dissection was then carried medial to the common carotid artery to locate the esophagus and trachea. The esophagus was divided and the upper end was reflected rostrally to expose the paired PCA muscles. The dissection was bathed in mineral oil to prevent drying. The marginal mandibular branch of the facial nerve was exposed for bipolar stimulation through a separate cheek incision.

A tracheal cannula was inserted and the rat was paralyzed with pancuronium bromide (1.0 mg/kg) and artificially ventilated with oxygen-enriched air. End-tidal carbon dioxide level and endotracheal pressure were monitored. Additional doses of pancuronium (0.5 mg/kg) were administered as required to maintain neuromuscular blockade after establishing that there was no response to noxious stimuli.

The preparation was mounted in a stereotaxic frame that enabled fixation of the skull and spinal

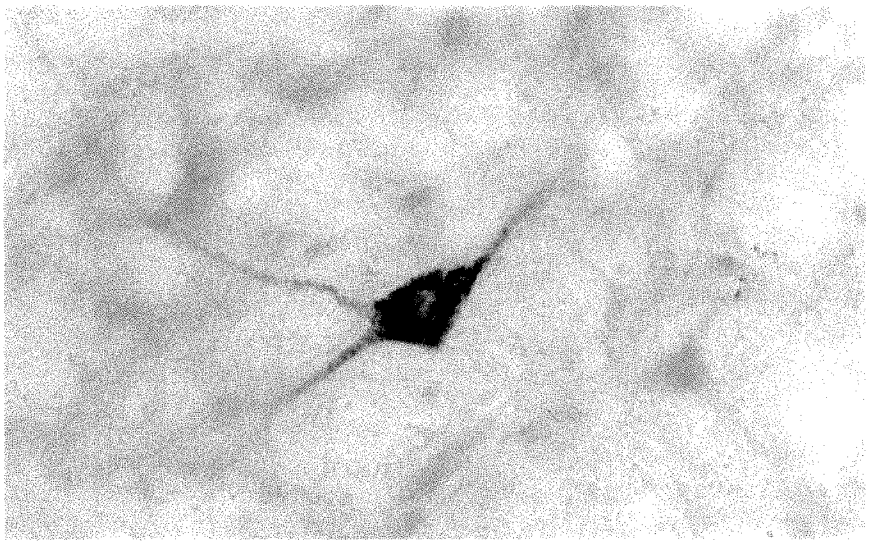

Fig 1. Photomicrograph of single posterior cricoarytenoid motoneuron retrogradely labeled by cholera toxin B subunit.

column, and bilateral pneumothoraces were made to minimize transmitted respiratory movement. A posterior fossa craniectomy was performed together with removal of a portion of the parietal and occipital bones to expose the brain stem. A cervical laminectomy from C1 to C6 was carried out to limit movement of the brain stem during recording. The dura was opened.

Electrophysiology. Phrenic nerve discharge was amplified (bandwidth 100 Hz to 3 kHz), full wave rectified, and integrated (time constant 50 milliseconds). Mapping of the antidromic field was carried out with glass microelectrodes containing 3-mol/L sodium chloride (resistance 1 MΩ) controlled by a PM20H piezo-hybrid manipulator (Bachofer, Germany). The signal was averaged after 20 to 100 sweeps. A CED1401 data acquisition system (CED, Cambridge, England) was used and data were analyzed and pictorially represented with Spike 2 (CED) and CorelDRAW! (Corel, Canada), respectively.

The location of the caudal border of the facial nucleus was mapped by recording the ipsilateral antidromic field potential obtained by bipolar stimulation of the marginal mandibular branch of the facial nerve to serve as a reference point for rostrocaudal localization of the PCA motoneurons. The PCA motoneurons were mapped by recording the ipsilateral field potential following focal bipolar stimulation (4 to 7 V) of the PCA muscle. In addition to rostrocaudal orientation, the position of the extracellular field was also described by the lateral distance of the electrode from the midline, and depth with respect to the dorsal surface of the brain stem. Recordings of the PCA motoneuron field potential were made at multiple sites to obtain a 3-dimensional grid, by varying each recording 1 dimension at a time by 100 μm. The criteria used to confirm antidromic activation of neurons were a fixed latency response in an all-or-none

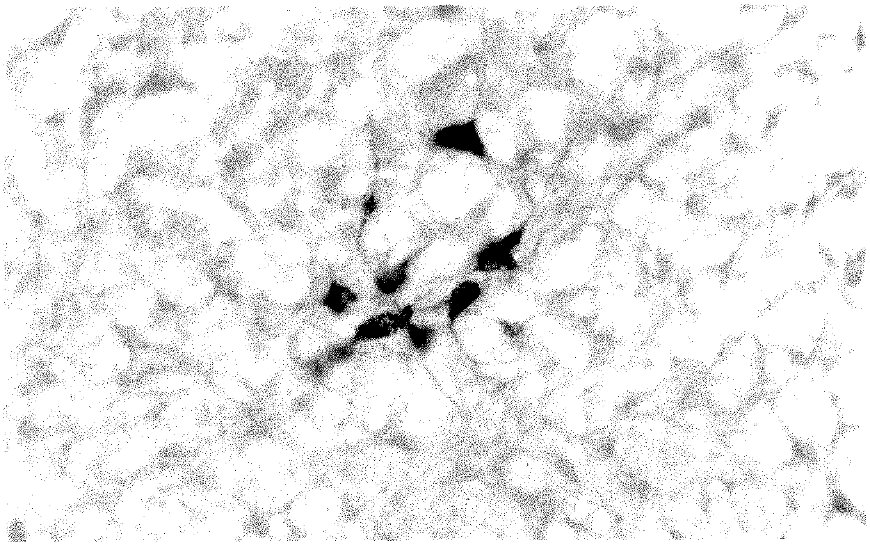

Fig 2. Photomicrograph of multiple posterior cricoarytenoid motoneurons retrogradely labeled by cholera toxin B subunit.

TABLE 1. VALUES FOR CAUDAL DISTANCE, LATERAL DISTANCE, AND DEPTH OF CTB-LABELED PCA MOTONEURONS

Location	*Mean ± SD*	*Range*
Caudal	1.47 ± 0.42	0.6-2.4
Lateral	1.51 ± 0.10	1.2-1.7
Depth	2.00 ± 0.17	1.5-2.3

Location of labeled neurons is described as distance caudal to caudal pole of facial nucleus, distance lateral to midline, and depth with respect to dorsal surface of medulla. Values are expressed in millimeters.

CTB — cholera toxin B subunit, PCA — posterior cricoarytenoid.

manner, a triphasic (positive-negative-positive) field, and collision of antidromic spikes with spontaneous discharges. Fields were regarded as being representative of the site of PCA motoneurons if the voltage was ≥20% of the maximum baseline shift recorded for that animal.

The animal experimentation in this study was approved by the Animal Ethics Committee, Royal North Shore Hospital, St Leonards, New South Wales.

RESULTS

RETROGRADE LABELING

A total of 194 neurons were labeled. In the 2 rats in which the contralateral vagus and superior laryngeal nerves were not divided, 16 neurons were ipsilateral to the side of PCA injection and 3 were contralateral. All 175 labeled neurons in the 4 rats in which the contralateral vagus and superior laryngeal nerves were divided were ipsilateral.

The neurons were multipolar, with dimensions of 33.2 ± 6.4 µm (mean ± standard deviation) in length and 22.4 ± 3.4 µm in width. Examples of labeled single and multiple neurons are shown in Figs 1 and 2, respectively. Locations of the labeled neurons are listed in Table 1.

ANTIDROMIC ACTIVATION

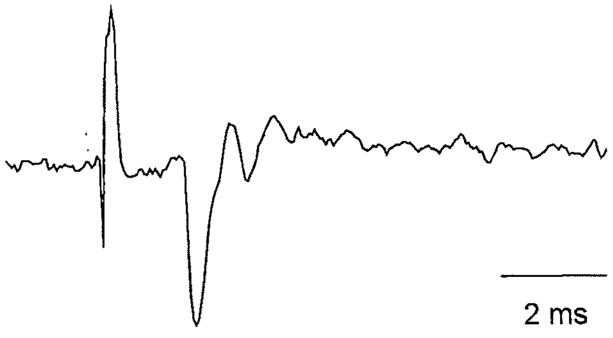

Fig 3. Example of extracellular field recording made following focal stimulation of PCA muscle.

The range over which extracellular antidromic field potentials were recorded following focal stimulation of the PCA muscle in 5 rats, and the site at which the largest field was obtained are listed in Table 2. An example of an extracellular antidromic field recording is shown in Fig 3.

The latency was measured for both the onset and conclusion of the antidromic response. Details regarding the latency and conduction velocity are listed in Table 3. Estimation of the conduction velocity was based on distances along the recurrent laryngeal nerve from the PCA muscle to the motoneuron of 125 mm on the left and 105 mm on the right. The conduction velocity ranged from 37.0 ± 5.8 (mean ± standard deviation) to 68.6 ± 5.0 m/s.

DISCUSSION

Retrograde labeling of PCA motoneurons in the rat has previously been reported,[25,31,34,39] and a summary of the findings is presented in Table 4 to serve as a comparison with the results obtained in this study using CTB.

In this study, the caudal pole of the facial nucleus was used as the rostrocaudal reference point for PCA motoneurons rather than the obex, to enable the results to be compared with those obtained by electrophysiological studies, where the facial nucleus regularly serves as a landmark and point of orientation

TABLE 2. LOCATION OF EXTRACELLULAR FIELD POTENTIAL RECORDED FOLLOWING FOCAL PCA MUSCLE STIMULATION

Animal	*Side*	*Caudal: Range*	*Lateral: Range*	*Depth: Range*	*Caudal: Maximum*	*Lateral: Maximum*	*Depth: Maximum*
1	L	0.5-1.5	1.4-2.0	1.7-2.4	1.1	1.7	2.2
2	R	0.8-2.0	1.5-2.1	1.7-2.3	1.5	1.7	1.9
3	R	0.8-1.8	1.5-1.9	2.0-2.4	1.5	1.7	2.2
4	L	0.9-1.7	1.5-2.1	1.8-2.5	1.5	1.7	2.2
5	R	1.1-1.7	1.5-1.9	2.1-2.3	1.5	1.8	2.2
Mean (SD)		0.8 (0.2)-1.7 (0.2)	1.5 (0.1)-2.0 (0.1)	1.9 (0.2)-2.4 (0.1)	1.4 (0.2)	1.7 (0.1)	2.1 (0.1)

Maximum values represent site in which largest field was recorded. Location of neurons is described as distance caudal to caudal pole of facial nucleus (caudal), distance lateral to midline (lateral), and depth with respect to dorsal surface of medulla (depth). Values are expressed in millimeters.

TABLE 3. LATENCY OF ANTIDROMIC RESPONSE AND ESTIMATED CONDUCTION VELOCITY

Animal	*Side*	*Latency: Onset (ms)*	*Latency: Conclusion (ms)*	*Conduction Velocity: Range (m/s)*
1	L	1.6	2.8	78-45
2	R	1.6	3.0	66-35
3	R	1.6	2.8	66-38
4	L	2.0	3.9	63-32
5	R	1.5	3.0	70-35

for medullary neurons. Reproducibility of data is greater compared with a reference point on the surface of the medulla in electrophysiological studies, because use of the caudal pole of the facial nucleus minimizes the variability resulting from rostrocaudal angulation of electrode insertion away from the plane perpendicular to the surface of the medulla.

The obex is approximately 2.6 mm caudal to the caudal pole of the facial nucleus.[59] This study is therefore in agreement with the findings of Portillo and Pasaro,[31] who used fluorescent dyes to localize PCA motoneurons immediately rostral to the obex. There is, however, an overall lack of uniformity between the results reported in the various studies of PCA motoneurons in rats using retrograde labeling techniques, with regard to both rostrocaudal location and neuron morphology. This could be explained in part by the differences in physical properties of the retrograde tracers used, particularly the variability of uptake at the motor end plate, and the possibility of spread to adjacent muscles.

There has been inconclusive evidence from retrograde tracer studies supporting bilateral representation of PCA and other laryngeal motoneurons in rats. Fay et al,[39] using pseudorabies virus, demonstrated bilateral labeling of PCA motoneurons in a study in which they removed the superior cervical ganglia bilaterally to prevent spread along laryngeal sympathetic fibers. Karim and Leong[60] reported bilateral nucleus ambiguus labeling following immersion of the cut end of the left cervical vagus nerve in horseradish peroxidase. Both Pasaro et al[26] and Lobera et al[61] found additional labeling of the contralateral nucleus ambiguus following injection of the laryngeal muscles with horseradish peroxidase despite division of the contralateral recurrent laryngeal nerve. In all of these studies, dye spillage or contralateral spread of dye within the larynx conveyed along the contralateral recurrent or superior laryngeal nerve may have been responsible for the contralateral motoneuron staining observed.

In this CTB study, 3 of 19 labeled neurons were contralateral, but in the subsequent experiments in which the contralateral cervical vagus and superior laryngeal nerves were divided, all 175 labeled neurons were ipsilateral to the side of the PCA muscle to which CTB was applied. This strongly suggests a purely ipsilateral brain stem representation for the PCA muscle in the rat.

Data regarding the dimensions of PCA motoneurons in the rat displayed in Table 4 do not show any uniformity. The findings in this study are, however, comparable with those reported by Saxon et al[62] for retrogradely labeled laryngeal motoneurons in the rat; they found dimensions of 41.7 ± 7.7 μm (mean ± standard deviation) in the long axis and 30.2 ± 6.4 μm in the short axis.

While retrograde labeling studies provide valuable information, problems related to the application and uptake of dyes can be avoided and more detailed information obtained by employing electrophysiological techniques for identification of PCA motoneurons.

The dorsal approach to the larynx described combines exposure of the PCA muscle for focal stimulation together with access via a posterior fossa craniectomy to the brain stem for recording of the antidromic field generated. Reproducible data were obtained regarding the location and distribution of the PCA

TABLE 4. RETROGRADE TRACER STUDIES OF PCA MOTONEURONS IN RAT

	Hinrichsen and Ryan[25]	*Portillo and Pasaro[31]*	*Flint et al[34]*	*Fay et al[39]*
Retrograde tracer	Horseradish peroxidase	Diamidino yellow and true blue	Fast blue	Pseudorabies virus
Rostrocaudal distribution	Main peak immediately caudal to obex; another small peak 0.5 mm caudally	Obex to 1.3 mm rostrally	From 0.6 mm rostral to obex to 1.6 mm caudal to obex	Mid nucleus ambiguus
Ipsilateral or bilateral representation	Ipsilateral	Ipsilateral	Ipsilateral	Additional contralateral representation
Neuron morphology	Two cell types: larger multipolar neurons and smaller neurons with few dendrites	15- to 25-μm-diameter multipolar neurons		50-μm-diameter multipolar neurons

TABLE 5. COMPARISON OF LOCATION OF PCA MOTONEURON POOL OBTAINED BY CTB RETROGRADE LABELING AND EXTRACELLULAR FIELD MAPPING FOLLOWING ANTIDROMIC ACTIVATION

	Caudal		*Lateral*		*Depth*	
	Maximum	*Range*	*Maximum*	*Range*	*Maximum*	*Range*
CTB labeling	1.5 (0.4)	0.6-2.4	1.5 (0.1)	1.2-1.7	2.0 (0.2)	1.5-2.3
Extracellular field	1.4 (0.2)	0.8-1.7	1.7 (0.1)	1.5-2.0	2.1 (0.1)	1.9-2.4

Location is described as caudal distance from caudal pole of facial nucleus (caudal), lateral distance from midline (lateral), and depth with respect to dorsal surface of brain stem (depth). Values are expressed in millimeters with SD in parentheses. Term maximum represents mean of values in CTB study, and site of maximum recorded field in extracellular study.

motoneuron pool in the ventrolateral medulla of the rat, and the results are similar to those obtained by retrograde labeling with CTB. A comparison of results is shown in Table 5.

Morphological and electrophysiological data obtained from studies of antidromically activated laryngeal motoneurons following recurrent laryngeal nerve stimulation are summarized in Table 6.[48-50,52,53] Comparing the size of the antidromic field reported in these studies with that obtained following focal PCA muscle stimulation is difficult, because the former studies included the thyroarytenoid, lateral cricoarytenoid, and interarytenoid motoneurons in addition to PCA motoneurons. Another confounding factor is the difference in size of the species studied. A number of common features, however, exist, which include the elongated shape in the rostrocaudal direction, the rostrocaudal location with respect to the obex and caudal pole of the facial nerve, and the estimated conduction velocity. Similarities also exist with the medial-to-lateral and ventral-to-medial dimensions of the motoneuron pool reported by Barillot et al.[52]

Generation of an antidromic field by focal muscle stimulation rather than direct stimulation of the nerve to the PCA muscle may lead to current spread to adjacent muscles or nerves, as can also occur with application of retrograde tracers to muscle. Careful electrode placement within the PCA muscle based on knowledge of the intralaryngeal distribution of the recurrent laryngeal nerve, combined with a low level of muscle stimulation, allowed minimization of unwanted current spread to achieve reproducible and consistent results.

An extracellular antidromic field potential, which corresponds to the distribution of the PCA motoneuron pool demonstrated by retrograde labeling with CTB, can be reliably obtained in a rat model following focal PCA muscle stimulation.

Establishing the extracellular antidromic field potential following focal PCA muscle stimulation in the rat model at a relatively constant site will allow more detailed intracellular studies of the PCA motoneurons to be performed.

TABLE 6. STUDIES OF RECORDINGS MADE FROM ANTIDROMICALLY ACTIVATED LARYNGEAL MOTONEURONS FOLLOWING RECURRENT LARYNGEAL NERVE STIMULATION

	Delgado-Garcia et al[48]	*Yajima and Hayashi*[49]	*Yajima and Hayashi*[50]	*Barillot et al*[52]	*Barillot et al*[53]
Species	Cat	Rat	Rat	Cat	Cat
Recording	Extracellular single neuron	Extracellular single neuron	Extracellular single neuron	Intracellular single neuron	Intracellular single neuron
Latency (ms)	1.3-2.8	2.9 ± 0.6	1.91 ± 0.45 (branched axon); 2.10 ± 0.53 (unbranched axon)		
Estimated conduction velocity (m/s)	35-60	45 ± 12			58.1 ± 17.2 (inspiratory neuron); 56 ± 19.3 (expiratory neuron)
Site within nucleus ambiguus (ipsilateral)	0-2 mm rostral to obex; 2.75-4 mm lateral to midline; 3.5-4.75 mm deep to dorsal surface	Dorsal formation of ventral division of nucleus ambiguus		2.0 mm rostral to obex to 0.5 mm caudal to obex	2.0 mm rostral to obex to 0.5 mm caudal to obex; 3.3-3.9 mm lateral to midline; 3.3-3.8 mm deep to dorsal surface
Resting membrane potential (mV)					−65.7 ± 12.5 (inspiratory neuron); −59.1 ± 12.0 (expiratory neuron)

REFERENCES

1. Murakami Y, Kirchner JA. Respiratory movements of the vocal cords. An electromyographic study in the cat. Laryngoscope 1972;82:454-67.

2. Suzuki M, Kirchner JA. The posterior cricoarytenoid as an inspiratory muscle. Ann Otol Rhinol Laryngol 1969;78:849-64.

3. Sherrey JH, Megirian D. Respiratory EMG activity of the posterior cricoarytenoid, cricothyroid and diaphragm muscles during sleep. Respir Physiol 1980;39:355-65.

4. Orem J, Lydic R. Upper airway function during sleep and wakefulness: experimental studies on normal and anesthetized cats. Sleep 1978;1:49-68.

5. England SJ, Kent G, Stogryn HAF. Laryngeal muscle and diaphragm activities in conscious dog pups. Respir Physiol 1985;60:95-108.

6. Harding R, England SJ, Stradling JR, Kozar LF, Phillipson EA. Respiratory activity of laryngeal muscles in awake and sleeping dogs. Respir Physiol 1986;66:315-26.

7. Bartlett D. Effects of hypercapnia and hypoxia on laryngeal resistance to airflow. Respir Physiol 1979;37:293-302.

8. Mu L, Yang S. The role of the posterior cricoarytenoid muscle in phonation: an electromyographic investigation in dogs. Laryngoscope 1991;101:849-54.

9. van Lunteren E, Van de Graaff WB, Parker DM, et al. Activity of upper airway muscles during augmented breaths. Respir Physiol 1983;53:87-98.

10. van Lunteren E, Van de Graaff WB, Parker DM, et al. Nasal and laryngeal reflex responses to negative upper airway pressure. J Appl Physiol Respirat Environ Exercise Physiol 1984;56:746-52.

11. Scott SC, Inman JD, Moss IR. Ontogeny of sleep/wake and cardiorespiratory behavior in unanesthetized piglets. Respir Physiol 1990;80:83-102.

12. Praud JP, Kianicka I, Leroux JF, Dalle D. Laryngeal response to hypoxia in awake lambs during the first postnatal days. Pediatr Res 1995;37:482-8.

13. Brancatisano TP, Dodd DS, Engel LA. Respiratory activity of posterior cricoarytenoid muscle and vocal cords in humans. J Appl Physiol Respirat Environ Exercise Physiol 1984; 57:1143-9.

14. Eichenwald EC, Howell RG, Kosch PC, Ungarelli RA, Lindsey J, Stark AR. Developmental changes in sequential activation of laryngeal abductor muscle and diaphragm in infants. J Appl Physiol 1992;73:1425-31.

15. Duara S, Rojas M, Claure N. Upper airway stability and respiratory muscle activity during inspiratory loading in full-term neonates. J Appl Physiol 1994;77:37-42.

16. Duara S, Neto GS, Claure N. Role of respiratory muscles in upper airway narrowing induced by inspiratory loading in preterm infants. J Appl Physiol 1994;77:30-6.

17. Kuna ST, Smickley JS, Insalaco G. Posterior cricoarytenoid muscle activity during wakefulness and sleep in normal adults. J Appl Physiol 1990;68:1746-54.

18. Kuna ST, Day RA, Insalaco G, Villeponteaux RD. Posterior cricoarytenoid activity in normal adults during involuntary and voluntary hyperventilation. J Appl Physiol 1991;70: 1337-85.

19. Kosch PC, Hutchison AA, Wozniak JA, Carlo WA, Stark AR. Posterior cricoarytenoid and diaphragm activities during tidal breathing in neonates. J Appl Physiol 1988;64:1968-78.

20. Bieger D, Hopkins DA. Viscerotopic representation of the upper alimentary tract in the medulla oblongata in the rat: the nucleus ambiguus. J Comp Neurol 1987;262:546-62.

21. Szentagothai J. Die Lokalisation der Kehlkopfmuskulatur in den Vaguskernen. Z Anat Entwicklungsgesch 1943;112:704-10.

22. Lawn AM. The localization in the nucleus ambiguus of the rabbit of the cells of origin of motor nerve fibers in the glossopharyngeal nerve and various branches of the vagus nerve by means of retrograde degeneration. J Comp Neurol 1966;127: 293-306.

23. Gacek RR. Localization of laryngeal motor neurons in the kitten. Laryngoscope 1975;85:1841-60.

24. Yoshida Y, Miyazaki T, Hirano M, Shin T, Kanaseki T. Arrangement of motoneurons innervating the intrinsic laryngeal muscles of cats as demonstrated by horseradish peroxidase. Acta Otolaryngol (Stockh) 1982;94:329-34.

25. Hinrichsen CF, Ryan AT. Localization of laryngeal motoneurons in the rat: morphologic evidence for dual innervation? Exp Neurol 1981;74:341-55.

26. Pasaro R, Lobera B, Gonzalez-Baron S, Delgado-Garcia JM. Localization of laryngeal muscle motoneurons in rat. Rev Esp Fisiol 1981;37:317-22.

27. Pasaro R, Lobera B, Gonzalez-Baron S, Delgado-Garcia JM. Cytoarchitectonic organization of laryngeal motoneurons within the nucleus ambiguus of the cat. Exp Neurol 1983;82: 623-34.

28. Davis PJ, Nail BS. On the location and size of laryngeal motoneurons in the cat and rabbit. J Comp Neurol 1984;230:13-32.

29. Yoshida Y, Mitsumasu T, Hirano M, Kanaseki T. Somatotopic representation of the laryngeal motoneurons in the medulla of monkeys. Acta Otolaryngol (Stockh) 1985;100:299-303.

30. Basterra J, Chumbley C, Dilly PN. Topographic distribution of laryngeal motor neurons in the nucleus ambiguus of the guinea pig studied by horseradish peroxidase (HRP) technique. Acta Otolaryngol (Stockh) 1987;103:105-10.

31. Portillo F, Pasaro R. Location of motoneurons supplying the intrinsic laryngeal muscles of rats. Horseradish peroxidase and fluorescence-labeling study. Brain Behav Evol 1988;32: 220-5.

32. Nahm I, Watanabe H, Maeyama T, Morikawa I, Shin T. Localization of laryngeal motoneurons in the medulla oblongata of guinea pigs. Auris Nasus Larynx 1989;16:117-26.

33. Kitamura S, Okubo J, Ogata K, Sakai A. Fibers supplying the laryngeal musculature in the cranial root of the rabbit accessory nerve: nucleus of origin, peripheral course and innervated muscles. Exp Neurol 1987;97:592-606.

34. Flint PW, Downs DH, Coltrera MD. Laryngeal synkinesis following reinnervation in the rat. Neuroanatomic and physiologic study using retrograde fluorescent tracers and electromyography. Ann Otol Rhinol Laryngol 1991;100:797-806.

35. Hisa Y, Sato F, Fukui K, Ibata Y, Mizuokoshi O. Nucleus ambiguus motoneurons innervating the canine intrinsic laryngeal muscles by the fluorescent labeling technique. Exp Neurol 1984;84:441-9.

36. Okubo J, Kitamura S, Ogata K, Sakai A. Localization of rabbit laryngeal motoneurons in the nucleus ambiguus. Exp Neurol 1987;96:528-39.

37. Portillo F, Pasaro R. Location of bulbospinal neurons and of laryngeal motoneurons within the nucleus ambiguus of the rat and cat by means of retrograde fluorescent labelling. J Anat 1988;159:11-8.

38. Holtman JR Jr. Immunohistochemical localization of serotonin- and substance P–containing fibers around respiratory muscle motoneurons in the nucleus ambiguus of the cat. Neuroscience 1988;26:169-78.

39. Fay R, Gilbert KA, Lydic R. Pontomedullary neurons transsynaptically labeled by laryngeal pseudorabies virus. Neuroreport 1993;5:141-4.

40. Arita H, Sakamoto M, Hirokawa Y, Okado N. Serotonin innervation patterns differ among the various medullary motoneuronal groups involved in upper airway control. Exp Brain Res 1993;95:100-10.

41. Lipski J. Antidromic activation of neurones as an analytical tool in the study of the central nervous system. J Neurosci Methods 1981;4:1-32.

42. Eccles JC. The central action of antidromic responses in motor nerve fibers. Pflügers Arch Ges Physiol Menschen Tiere 1955;260:385-415.

43. Brock LG, Coombs JS, Eccles JC. Intracellular recording from antidromically activated motoneurones. J Physiol (Lond) 1953;122:429-61.

44. Fuortes MGF, Frank K, Becker MC. Steps in the production of motoneuron spikes. J Gen Physiol 1957;40:735-52.

45. Phillips CG. Actions of antidromic pyramidal volleys on single Betz cells in the cat. Q J Exp Physiol 1959;44:1-25.

46. Serra R, Ribas J, Canedo A, Gonzalez-Baron S, Delgado-Garcia JM. Field potentials induced in the ambiguus nucleus by stimulation of the recurrent and superior laryngeal nerves. Rev Esp Fisiol 1982;38:367-73.

47. Delgado-Garcia JM, Lopez-Barneo J, Rial R, Gonzalez-Baron S. Functional activity of the laryngeal motor neurons in the anesthetized cat. Rev Esp Fisiol 1982;38:375-81.

48. Delgado-Garcia JM, Lopez-Barneo J, Serra R, Gonzalez-Baron S. Electrophysiological and functional identification of different neuronal types within the nucleus ambiguus in the cat. Brain Res 1983;277:231-40.

49. Yajima Y, Hayashi Y. Identification of motoneurons in the nucleus ambiguus by antidromic stimulation of the superior and recurrent laryngeal nerves in rats. Brain Res 1983;288:302-6.

50. Yajima Y, Hayashi Y. Electrophysiological evidence for axonal branching of ambiguus laryngeal motoneurons. Brain Res 1989;478:309-14.

51. Fenik V, Kubin L, Okabe S, Pack AI, Davies RO. Differential sensitivity of laryngeal and pharyngeal motoneurons to iontophoretic application of serotonin. Neuroscience 1997;81:873-85.

52. Barillot JC, Bianchi AL, Gogan N. Laryngeal respiratory motoneurons: morphology and electrophysiological evidence of separate sites for excitatory and inhibitory synaptic inputs. Neurosci Lett 1984;47:107-12.

53. Barillot JC, Grelot L, Reddad S, Bianchi AL. Discharge patterns of laryngeal motoneurons in the cat: an intracellular study. Brain Res 1990;509:99-106.

54. Bras H, Destombes J, Gogan P, Tyc-Dumont S. The dendrites of single brain-stem motoneurons intracellularly labelled with horseradish peroxidase in the cat. An ultrastructural analysis of the synaptic covering and the microenvironment. Neuroscience 1987;22:971-81.

55. Yajima Y, Hayashi Y. GABA(A) receptor–mediated inhibition in the nucleus ambiguus motoneuron. Neuroscience 1997;79:1079-88.

56. Takeda R, Haji A. Electrophysiological properties of respiratory laryngeal motoneurons and effects of thiopental. Tohoku J Exp Med 1988;156(suppl):21-31.

57. Berkowitz RG, Sun Q-J, Chalmers J, Pilowsky PM. Respiratory activity of the rat posterior cricoarytenoid muscle. Ann Otol Rhinol Laryngol 1997;106:897-901.

58. Llewellyn-Smith IJ, Minson JB, Pilowsky PM. Retrograde tracing with cholera toxin B–gold or immunocytochemically-detected cholera toxin B in the central nervous system. In: Conn PM, ed. Neurotoxins. Methods in neuroscience. Vol 8. New York, NY: Academic Press, 1992:180-201.

59. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 2nd ed. San Diego, Calif: Academic Press, 1986.

60. Karim MA, Leong SK. Neurons of origin of cervical vagus nerves in the rat and monkey. Brain Res 1980;186:208-10.

61. Lobera B, Pasaro R, Gonzalez-Baron S, Delgado-Garcia JM. A morphological study of ambiguus nucleus motoneurons innervating the laryngeal muscles in the rat and cat. Neurosci Lett 1981;23:125-30.

62. Saxon DW, Robertson GN, Hopkins DA. Ultrastructure and synaptology of the nucleus ambiguus in the rat: the semicompact and loose formations. J Comp Neurol 1996;375:109-27.

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THE OTOLARYNGOLOGIST AS A ROLE MODEL

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Throughout our daily interactions with medical students and residents, each of us, whether we like it or not, functions as a role model. Since role modeling is primarily a passive function — teaching by example — we may not be acutely aware of this role and its importance. In what respect is the concept of the role model important to otolaryngology–head and neck surgery? In addition to the function of specific training — teaching the trade — most of the literature on role modeling cites 2 major areas of significance: 1) influencing medical students' career choices and 2) facilitating socialization into the world of medicine with the establishment of an appropriate professional identity. This brief article reviews some of the current literature, catalogs those attributes that have been identified as those of excellent role models, and offers some thoughts as to what our specialty might consider in response to the challenges to medical education in the changing health care environment.

KEY WORDS — education, otolaryngology, residency, role model, teaching.

INTRODUCTION

Training and Education: From Role Model to Mentor. William Osler established the tradition of clinician-educator in North America in the outpatient department at Johns Hopkins University.[1,2] The system of learning from cases under the directorship of a professor and his staff apparently replaced a loose apprenticeship system and was consistent with the Flexner report.[3] Osler's system remains, albeit influenced by the pressures of the changing health care environment, its remarkable strength being core virtues that are principles of adult learning (which is collaborative, involves actively solving problems, and is readily applicable to future work).[3]

Role Model. Residents and students must see a standard of excellence to pursue, a model to copy. The ideal role model is a superior clinician who is a dedicated to teaching. The key concept is dedication, taking teaching seriously. The role model helps to facilitate learning as the student attempts to emulate the instructor. Role modeling is intrinsic to the process of teaching but not to the substance of teaching; it helps to instill within the learner a desire to gain new knowledge and learn to apply that knowledge.[4]

The ability of the role model to stimulate intellectual curiosity is an essential element of good role modeling.[4] Faculty members who demonstrate the importance of scientific reasoning and continuing self-education convey the message that medicine is constantly evolving.[5] Some students are not accustomed to learning through the scientific process, although it is fundamental to medical research and clinical competence; instructors who allow students to absorb information passively without encouraging critical analysis and inquiry provide a disservice to students.[4]

Giving liberal doses of systematic feedback is one of the most effective teaching tools. But role models must exhibit patience, providing students with factual, descriptive comments in a supportive, sensitive manner, lest students misconstrue this feedback as a personal attack rather than a deliberate teaching technique.[6]

In otolaryngology–head and neck surgery, role modeling occurs in 5 milieus: the classroom, the laboratory, the ambulatory setting, the patient bedside, and the operating room. A role model in the classroom and laboratory can demonstrate the importance of the scientific thought process and foster a skepticism of dogma. Hospital rounds and the ambulatory setting are the most obvious situations in which role modeling occurs, as students observe appropriate behavior and the physician-patient interaction.[4] The operating room is where focus of attention, technical skills, and meticulous attention to details are demonstrated.

Preceptorship is based on principles of adult education. Adult education 1) is problem-centered, 2) is conducted in a supportive environment, 3) is experience-oriented, 4) is active, not passive, and 5) includes feedback, focusing on behavior, not the individual. Preceptorial relationships are especially useful in the developing of practical skills. The preceptor works either individually or with small groups of

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students and serves as a teacher, role model, and evaluator. As is typical of most role modeling, preceptorial relationships are relatively short.[7]

Mentor. Mentoring is role modeling taken to a higher level. What the role model is for medical students, the mentor is what we should be for our residents. The effective mentor must be an exemplary role model. Mentoring involves an intense global and long-term relationship. It encompasses both professional and personal domains, and may span several years or beyond the period of the structured mentorship. The mentor serves as a teacher, role model, coach, and confidante, and works one-on-one to achieve various goals.[7]

THE FUNCTION OF THE ROLE MODEL

Career Choice. The first function of role modeling — career choice — is important to us as specialists in our relationships with medical students. Role models do not believe they intentionally try to recruit students, but instead feel that demonstrating enthusiasm and a sincere love for what they do has a strong influence toward this end. Interestingly, most of the recent literature about role modeling has been written by and for educators working in the primary care specialties.[8] Many studies are designed to gather data to be used for the purpose of recruiting medical students into primary care areas. In this regard, it is particularly important for medical students to be exposed to otolaryngologists who are excellent role models.

And the earlier, the better. Forty-eight percent of medical students change their career plans during their second year.[9] Waiting until the third year to expose medical students to otolaryngology–head and neck surgery may simply be too late. In fact, one study showed the strongest predictor of final career choice was career choice at baseline.[9] This indicates the importance of selecting medical students who are already inclined toward a specific career. The data are inconsistent, however; another study reported that the majority of students made final decisions about specialties during the fourth year.[10] Indeed, some authors conclude that role models will have little influence on many students' career decisions.[11] Some students describe behaviors and personalities of attending physicians whom they admired and hoped to emulate in their own, different fields of choice (a reflection of the socialization function of role models).

Negative role models, in contrast, had strong dissuasive affects on specialty selections; attributes of these individuals included difficult personalities, perceived lack of camaraderie, professional dissatisfaction, and disheartening physician-patient interactions. Overworked and dissatisfied physicians who demonstrate poor communication skills with patients and students may permanently close career options. They are a reminder of the importance of effective training and development of faculty and residents as teachers.[11]

Socialization Into the Medical Profession. The second function of role modeling is of importance for both medical students and residents: socialization into the medical profession and the establishment of a professional identity, facilitating the acquisition of the values, attitudes, and behavior associated with professionalism, humanism, and ethical practice.[3,5,8] The implication is that role modeling can actually function to modify behavior. Residents in otolaryngology–head and neck surgery need excellent role models and mentors to fulfill this need.

A student's capacity for compassion can be positively or negatively influenced by role models. Students tend to have less and less altruism as they proceed through medical training.[4] Patient management in a highly technological medical center is seen as being fragmented and as having a lack of interest in total patient care; this results in far more emphasis on tertiary care and less attention on teaching continuity of care and the treatment of chronic illnesses.[4]

ATTRIBUTES OF THE EXCELLENT ROLE MODEL

Given the importance of role models, it is necessary to identify specific attributes of excellent physician role models. The effective positive role model purposefully acquires teaching skills, and is responsive to the complex elements involved when interacting with learners.[3]

Wright et al[8] undertook a comprehensive multicenter study to identify attributes of the excellent role model. Their study, as is typical of such studies, identifies these attributes by asking members of the internal medicine house staff to name physicians whom they consider to be excellent role models. The following were identified as attributes of excellent role models:

1. Spending more than 25% of one's time teaching.
2. Spending 25 or more hours per week teaching and conducting rounds when serving as attending physician.
3. Stressing the importance of the doctor-patient relationship.
4. Teaching the psychosocial aspects of medicine.
5. Having served as chief resident or having participated in formal training in teaching (including faculty development programs or teaching work-

shops).

In addition to these 5 attributes, the study made several other observations of interest to otolaryngologists. Generalists were more likely to be named as excellent role models than subspecialists. The study also found that excellent role models enjoyed teaching medical students and residents more than did controls and were more satisfied with medicine as a career. Engaging in more activities that build relationships with house officers (such as organizing a dinner, sharing professional experiences, talking about their personal lives, and trying to learn about the lives of house staff) was associated with increased likelihood of being recognized as an excellent role model. Dunnington[12] points out that effective mentors recognize students as individuals with private lives; they have spouses and significant others and often children, mortgage payments, and family emergencies.

Attending physicians who were identified as excellent role models committed a greater percentage of their efforts to teaching, administration, and curriculum development.[8] They think about student issues and assess learning needs.[12] In contrast, a greater proportion of time spent on research or clinical work was not associated with being identified as an excellent role model. In fact, career achievements related to research — including total publications and time spent on research — were inversely associated with identification as an excellent role model. The authors speculated that the most important factor preventing attending physicians who concentrate on research from being identified as an excellent role model may be the lack of contact with house staff, and such exposure is prerequisite to becoming an effective role model.[8]

Characteristics of physician role models highlighted in other studies[13,14] included personal qualities (such as compassion, sense of humor, and integrity), clinical skills (such as proficiency as a diagnostician and effective interaction with patients and their families), and teaching skills (such as a nonthreatening style and the ability to explain difficult subjects). Students ranked personality as first in importance, followed by clinical skills and competence, teaching ability, area of specialty, research experience and publications, and position of academic rank.[13]

In another study, the most important characteristics of role models included enthusiasm for the specialty, honesty, being comfortable with ignorance,[15] and actively involving and communicating effectively with students. Enthusiasm was the characteristic that role models felt to be most important in influencing students' attitudes.[15] Other positive factors include self-criticism, assuming responsibility, recognizing limitations, respect for and sensitivity toward patients and trainees, and a wholesome sense of humor.[16,17]

Most publications on the subject state that a role model should ensure a supportive environment for learning.[12] Too often I have observed the inappropriate behavior of autocratic surgeons who demean and humiliate students, residents, and even fellows, apparently with the purpose of stimulating learning. That is not to say, however, that role models should not ask questions. Indeed, effective role models are skilled in questioning. The skilled questioner focuses on a higher level of thinking and poses questions that call for comparison, analysis, and reasoning. Good questioning is the difference between giving students the information and provoking their critical thinking until they are able to come to a discovery on their own.[12] Questioning also functions to evaluate students. "We assess knowledge by asking questions, we assess skills by observation, and we assess attitudes by professional intimacy. Without such a needs assessment we will teach facts that are already known, train for skills that have already been acquired, and inspire values that are already shared."[18(p125)]

FUTURE DIRECTIONS

What are the implications of these data at a time when faculty members are under increasing pressure to generate more revenue, and when major medical schools prioritize basic medical research in the face of decreasing peer-reviewed funding? It has been noted that medical education is no longer the major activity of American medical schools.[19] Addressing this problem involves re-framing institutional goals, making education of students once again the highest priority of medical schools; it requires a change in the basic culture of academic medical centers and restructuring financial resources.[20]

Career Choice. Because role modeling influences career choice, and because it is important for socialization into the medical profession, increased support of excellent role models is imperative. It is indeed fortunate that many of the attributes of excellent role models are skills that can be acquired or behavior that can be modified.[8] The costs of this faculty development — in time and money — need to be supported so as to help physicians acquire the skills required for effective, positive role modeling.[8] In this context, it is notable that mentoring and role modeling has been found to be only the third most important factor in decisions about promotion of clinical educators (after teaching skills and clinical skills).[6]

To influence medical student recruitment into otolaryngology, faculty and house staff must demonstrate enthusiasm about our specialty and about teaching, and love for our profession.[15] We can also make opportunities for contact with first- and second-year medical students, such as by teaching anatomy of the head and neck in the first-year gross anatomy laboratory.

Socialization. Residents need to be made aware of their influence as role models, since students anticipate their advancement to that level. The majority of residency programs do not specifically screen applicants for potential teaching ability, nor do programs provide instruction in teaching skills once the residents have been selected.[4]

Department chairmen and senior faculty members should conspicuously demonstrate positive behavior. In the clinical setting, the attitude of the attending physician can make a difference in how students are treated by residents.

Support for the Excellent Role Model. The medical school should reward good teaching and formally recognize faculty members and residents who are effective teachers and role models.[4] Attending physicians who are excellent role models need to be identified so that they can be encouraged and supported to spend more time with medical students and residents. Some would argue that they should also be rewarded with career advancements.[13]

Specific areas that require resources for adequate support include 1) programs to ensure keeping up-to-date with the medical literature, stressing an evidence-based approach; 2) developing teaching skills; 3) getting academic recognition, promotion, and tenure for clinician-educators, establishing guidelines and setting standards to reward clinician-educators for their scholarship, which is not limited to peer-reviewed publications, and for their multiple contributions to teaching; 4) sustaining career satisfaction; 5) working in the managed-care environment; and 6) attaining economic goals; physicians who teach currently work longer days to offset the expected income losses resulting from time spent teaching. Teaching generates no income, yet teaching is the central mission of every medical school and residency program.[3]

Senior faculty practitioners should be organized into cadres of role models and be given incentives to invest the time and effort needed to fulfill this part of their duties.[20] Those in leadership positions must acknowledge that role models may be negative as well as positive.[20]

I conclude with Tosteson's poignant observation that "the most important, indeed, the only, thing we have to offer our students is ourselves. Everything else they can read in a book."[21(p693)]

REFERENCES

1. Futcher TB. An address on the importance of bedside study and teaching. Can Med Assoc J 1935;32:357-64.
2. Osler W. The natural method of teaching the subject of medicine. JAMA 1901;36:1673-9.
3. Branch W, Kroenke K, Levinson W. The clinician-educator — present and future roles. J Gen Intern Med 1997;12 (suppl 2):S1-S4.
4. Ficklin FL, Browne VL, Powell RC, Carter JE. Faculty and house staff members as role models. J Med Educ 1988; 63:392-6.
5. Barondess J. The GPEP report. III. Faculty involvement. Ann Intern Med 1985;103:454-5.
6. Irby DM. Clinical teaching and the clinical teacher. J Med Educ 1986;61:35-45.
7. Sachdeva AK. Preceptorship, mentorship, and the adult learner in medical and health sciences education. J Cancer Educ 1996;11:131-6.
8. Wright SM, Kern DE, Kolodner K, Howard DM, Brancati FL. Attributes of excellent attending-physician role models. N Engl J Med 1998;339:1986-93.
9. Hunt DK, Badgett RG, Woodling AE, Pugh JA. Medical student career choice: do physical diagnosis preceptors influence decisions? Am J Med Sci 1995;310:19-23.
10. Lieu TA, Schroeder SA, Altman DF. Specialty choices at one medical school: recent trends and analysis of predictive factors. Acad Med 1989;64:622-9.
11. Mutha S, Takayama JI, O'Neil EH. Insights into medical students' career choices based on third- and fourth-year students' focus-group discussions. Acad Med 1997;72:635-40.
12. Dunnington GL. The art of mentoring. Am J Surg 1996; 171:604-7.
13. Wright S, Wong A, Newill C. The impact of role models on medical students. J Gen Intern Med 1997;12:53-6.
14. Wright SM. Examining what residents look for in their role models. Acad Med 1996;71:290-2.
15. Ambrozy DM, Irby DM, Bowen JL, Burack JH, Carline JD, Stritter FT. Role models' perceptions of themselves and their influence on students' specialty choices. Acad Med 1997;72: 1119-21.
16. Irby DM. Clinical teacher effectiveness in medicine. J Med Educ 1978;53:808-15.
17. Barnes RH. You have a good sense of humor. You should be a doctor. Pharos 1991(Fall):727-9.
18. Whitman N. Creative medical teaching. Salt Lake City, Utah: Department of Family and Preventative Medicine, University of Utah School of Medicine, 1990:125.
19. Abrahamson S. The state of American medical education. Teach Learn Med 1990;2:120-5.
20. Reuler JB, Nardone DA. Role modeling in medical education. West J Med 1994;160:335-7.
21. Tosteson DC. Learning in medicine. N Engl J Med 1979; 301:690-4.

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EDUCATION IN LARYNGOLOGY: RISING TO OLD CHALLENGES

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Education in laryngology has been a subject of interest at least since Louis Elsberg's address to the first meeting of the American Laryngological Association in 1879. Remarkable scientific, technological, and clinical advances during the 1980s and 1990s have elevated the standard of laryngological care. It is essential for training programs to promulgate these important advances through well-planned, comprehensive curricula. Such training should also foster an appreciation for the kinds of creative thought, interdisciplinary collaboration, and imaginative clinical practice that have been responsible for many of the recent dramatic advances in the field of laryngology.

KEY WORDS — education, fellowship training, laryngology, residency training, voice.

INTRODUCTION AND HISTORICAL PERSPECTIVE

As we approach the new millennium, it seems appropriate to ask whether education in laryngology and voice has kept pace with the remarkable clinical and technological advances of the last 2 decades. Throughout the history of otolaryngology, considerable time and thought have been devoted to educational issues. The founders of the American Laryngological Association (ALA) were deeply concerned with training in laryngology. This was the subject of Louis Elsberg's Presidential Address at the first meeting of the ALA on June 10, 1879, in New York.[1] Elsberg had begun teaching laryngology in 1861, and he established the first training clinic affiliated with any institution, in 1863, at the University of the City of New York. By 1879, when the ALA was formed, Elsberg reported that there were 25 laryngology teachers in American medical schools. Elsberg's extraordinary address (57 published pages) provides a fascinating review of the history of laryngology, and culminates with a section entitled "Laryngological Instruction." Elsberg reviewed the mission of laryngology, and of the ALA in particular, as being not only to develop greater knowledge and skills, but also to disseminate them and raise the standard of care and education. Elsberg's address to the second meeting of the ALA was delivered by Jacob Solis-Cohen, in Elsberg's absence.[2] Elsberg dedicated this entire address to specific training issues, including requirements for basic science, and clinical knowledge that should be acquired before physicians in training are permitted to care for patients. He was an advocate for both undergraduate and graduate education in laryngology and even proposed instruction in comparative laryngology to enhance the understanding of the human larynx through the study of other species. In his Presidential Address before the fourth meeting of the ALA, Frederick Knight emphasized the same concerns. He observed, "It will be a great gain when every physician feels he must own a laryngoscope. A more important point for us here arises, how we can make him use it intelligently; how all men who graduate from our medical schools shall be given a little available knowledge of laryngology during their precious time of pupilage."[3(p4)] Knight stressed the importance of both didactic education and laboratory and clinical training. He also recognized the expense of medical training and called for endowment of medical schools to support education, an uncommon notion in his day. In addition, Knight offered specific recommendations for the teaching of laryngology within the medical school curriculum, even courageously calling for an extension of the duration of medical study. In Birmingham, England, in 1890, John St Swithin Wilders addressed the section of laryngology and rhinology of the British Medical Association in "On the Teaching of Laryngology."[4] He, too, emphasized the importance of educating all physicians in the fundamentals of laryngology and use of the laryngoscope, and raised concerns about specialty hospitals, especially those that were not used by medical students in their education. He stressed the importance of laryngological education within general hospitals "which profess to educate students" and even raised an extremely controversial call "that no more special hospitals will be founded."[4(p377)]

Concerns about laryngology and its place in medi-

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cal education were highlighted again in Henry L. Swain's Presidential Address to the 23rd meeting of the ALA, in which he called for specific time within the medical school curriculum for courses in our field.[5] Since that time, training issues have been addressed sporadically. For example, in 1906, Knight[6] reviewed the state of training in laryngology, highlighting the proliferation of instrumentation. Reflecting back on Elsberg's day, Knight noted that "the meagre armamentarium offered in the olden time is in striking contrast with the vast collection of apparatus now at our command. It is not certain that the enormous multiplication of instruments in recent years brings marked advantage."[6(p840)] It would be interesting to hear his perspective if he were alive today amid technological developments that make his own armamentarium look meager indeed.

Thomas J. Harris[7] addressed the complex issues of postgraduate training in laryngology in an insightful paper read before the ALA in 1913. Residency programs as we know them had not yet been developed. Harris emphasized the importance of undergraduate preparation, trainee selection, basic science education (particularly anatomy and pathology), postgraduate courses, and supervised clinical experience. He also advocated the establishment of a minimum postgraduate training time for qualification in otolaryngology. Skillern, writing in *JAMA* in "Post-graduate Work in Laryngology," echoed these concerns, noting that "prior to 1918, practically no under-graduate teaching on a systematic and comprehensive scale had been successfully carried out in this country."[8(p1145)] Skillern observed that "the aspiring young laryngologist became of the opinion that once the submucous resection and the enucleation of the tonsil were mastered, he forthwith had become a full-fledged and competent specialist."[8(p1145)] At that time, a 6-week course was typical for specialty training in otolaryngology. He called for more rigorous and longer training in otolaryngology, and instruction in subspecialty areas such as laryngology and otology.

In the years following these early papers devoted to education in laryngology, only a few authors have returned to this important subject. Milligan[9] argued that there was insufficient time devoted to laryngology in the undergraduate medical school curriculum in Britain. Dean[10] devoted his 1925 ALA Presidential Address to the teaching of undergraduate laryngology, and Layton[11] addressed undergraduate and postgraduate education in 1940. In 1966, Alford[12] wrote an excellent review of the evolution of undergraduate and graduate medical training in the United States, the development of standards and quality control, and the effects of the evolving medical educational milieu on residency training in otolaryngology. Bailey[13] provided interesting insights into the early years of the introduction of laryngology as a component of otolaryngology training for medical students in the same issue of the *Laryngoscope* in which Alford's article was published. Thirty-three years later, the training of medical students in laryngology remains an important and challenging issue, as does the education of the general public on the importance of laryngological and related disorders; but they are beyond the scope of this paper, which is devoted to postgraduate training in laryngology. Currently, Medline lists more than 300 articles that address training in otolaryngology. However, specific laryngology and voice training issues are rarely mentioned in these publications, and when they are, they usually focus on the teaching of indirect laryngoscopy to medical students. Considering the historical importance of laryngology to the development of otolaryngology, it is surprising how little has been written about teaching this challenging subspecialty, especially in recent years.

In addition to concerns about curricular issues, laryngologists have been interested in the many practical aspects of teaching clinical laryngology. For example, before the advent of video-monitored laryngoscopy, a variety of devices was conceived to allow trainees to see the larynx during examinations performed by their mentors. Most involved the use of mirrors, such as Lukens' demonstroscope[14] in 1929 and a device developed at the Mayo Clinic around 1960 that attached a shortened laryngeal mirror to a headlight with a reflecting mirror.[15] It is also worth remembering that the laryngeal mirror itself was originally introduced as a training device, albeit for singers, by singing teacher Manuel Garcia in 1854.[16] A great many other clever devices have been introduced before and since that time that have enhanced diagnosis, treatment, and training in laryngology.[17] As early as 1936, Francis LeJeune reported that "the motion picture film has been found of inestimable value in teaching the younger student clinical pathology of the larynx."[18(p492)] LeJeune's fascinating report indicates that he not only recognized the educational value of motion pictures in teaching laryngoscopy and laryngeal surgery, but also that he learned from his studies. His careful observations of the larynx led him to advocate "carrying out sharp dissection with the laryngeal knife for the removal of the growth. Such a procedure usually ensures a smooth, straight cord when healed."[18(p492)] Nevertheless, because of the cumbersome equipment, the delay necessary to develop the film, and the time-consuming nature of motion picture laryngoscopy, this technology was not utilized widely in laryngology clinics. However, in the late 1950s and early 1960s, von Leden and Moore established a voice clinic and used high-speed motion pictures extensively in clinical care and laryngeal research.[19-22] They also described the importance of television as a teaching device in otolaryngology.[19] The application of an operating microscope to direct laryngoscopy improved not only surgical management but also training. Tardy[23] was among the first to highlight the importance of combining the microscope with a color television camera, in his 1972 article "Microscopic Laryngology: Teaching Techniques." He advocated television display of laryngeal surgery for the purpose of enhancing training by allowing everyone in the operating room to see what the surgeon was doing. In addition to describing the value of new technology (microscope-assisted laryngoscopy and color television monitoring), Tardy showed photo documentation of magnified laryngeal polyp resection using techniques considerably more delicate than the "stripping" technique popular at the time. This insightful article specifically listed the teaching value of televised microscopic laryngeal surgery and called for its use in residency programs. Although today Tardy's observations seem obvious, it should be remembered that at the time, some respected otolaryngologists and educational institutions still considered even the microscope (television monitoring aside) superfluous even for mastoid surgery, let alone laryngoscopy; and it was still believed by many that laryngoscopy could be performed perfectly well while holding an unsuspended laryngoscope with one hand and using instruments only in the other. Tardy's work was an important early step toward improving both physician education and patient care. Even so, it took a while to catch on. For example, at the University of Michigan, the first camera for the otolaryngology department's microscope was not purchased until 1978. Even then, it was ordered by Dr A. C. D. Brown of the department of anesthesia so that the anesthesiologist could see the surgical field during mastoid surgery, rather than by the otolaryngology department to enhance otological and laryngological training. In retrospect, such lengthy delay in acquiring television monitors from so venerable a training program seems hard to fathom. Hence, it behooves each of us entrusted with the training of young otolaryngologists to reflect upon whether we are guilty of perpetrating similar unfathomable delays at our own institutions today.

Since 1975 (in English in 1977), when Hirano[24] described the layered structure of the vocal fold, laryngology has enjoyed unprecedented growth. Great advances in our understanding of the anatomy and physiology of phonation have been paralleled by technological developments for voice quantification and outcomes assessment.[25] These advances have resulted in dramatic improvement in the standard of care for all patients with laryngological disorders,[26] and they have affected clinical care remarkably quickly, due largely to exceptional interdisciplinary collaboration. Stimulated by meetings such as the Voice Foundation's Annual Symposium on Care of the Professional Voice, founded by Wilbur James Gould, Hans von Leden, and others in 1972, laryngologists, speech-language pathologists, basic science researchers, singing teachers, acting teachers, performers, and others have worked together to advance knowledge and enhance patient care. They have developed a common language, posed questions of practical value in the clinic and studio, and developed an interdisciplinary paradigm for answering important questions through valid, reliable research. Much of this research has been reported at meetings of the ALA and of the American Broncho-Esophagological Association (ABEA), the Voice Foundation symposia, and dozens of other, similar meetings that have evolved over the last 2 decades. Because of the interdisciplinary nature of many of these meetings and research efforts, and the collaborative, penetrating discussions that occur among professionals of different disciplines at such meetings, even the most esoteric scientific advances are promulgated quickly. Their practical importance is probed at the time of their presentation, and new discoveries are applied to patient care throughout the world within days after such meetings end.

While the sheer amount of new information, equipment, diagnostic and therapeutic approaches, and surgical advancements is exciting, it has also created inconsistencies among training programs in the United States and throughout the world. Advances have been integrated piecemeal into various educational programs at different rates, and to different degrees. Now that laryngology and voice is well established as a subspecialty, it seems timely to reflect on what we have learned, what anyone finishing a residency in otolaryngology or a fellowship in laryngology and voice should be expected to know, and how we can best teach these essentials. The development and adoption of training guidelines for laryngology and voice should be encouraged not only to help program directors, but also to ensure reasonable consistency in minimal basic knowledge among graduates from all programs, with the end result being a consistently high level of care for laryngological patients. However, the rapid and successful evolution of our field highlights the need to consider more than just facts and skills as we develop training priorities. We should also try to impart an enthusiasm for the kind of interdisciplinary creativity that was responsible for our current evolution as a field and try to encourage and inspire similar academic and clinical creativity.

RESIDENCY TRAINING

While laryngology (including voice, speech, swallowing, and related disorders) constitutes only one segment of otolaryngology, it is particularly important, for several reasons. First, laryngological problems are common. Estimates of the incidence of voice disorders in school-age children range, for example, from 6% to 23%.[27] Although there are no reliable, valid data on the incidence of voice disorders in the adult population, they are probably as prevalent as they are in children; and they may be even more common in elderly adults, who are more likely to develop neurologic disorders with related voice, speech, and swallowing abnormalities. Most of our graduates will be called upon to care for patients with laryngological disorders and to educate colleagues (including primary care physicians) on the proper diagnosis and treatment of laryngeal problems. Second, the standard of care and state-of-the-art for management of voice disorders have changed dramatically and continuously throughout the 1980s and 1990s.[26,28] The diagnostic techniques, methods of documentation, and imperative for outcomes assessment that are now standard were nonexistent just a few years ago. Some surgical techniques that were routine in the 1970s are now considered negligent; and newer surgical techniques that replaced them in the 1980s are already obsolete. If we do not make a concerted effort to ensure that our residents are fully aware of these developments and their practical implications, then they may provide outdated treatment; and both they and their patients will suffer the consequences.

Providing such training is especially difficult in laryngology, because of the speed with which the field has developed. It is certainly not possible for all otolaryngologists, or even all academic otolaryngologists, to keep up with all developments in all fields within our specialty. There are many programs in which laryngology is managed and taught by general otolaryngologists, or by head and neck cancer surgeons without special training in the modern clinical and research aspects of laryngology. However, even if every training program in the United States had the desire and funds to hire an experienced and/or fellowship-trained laryngologist, there are not enough to supply all of the positions, yet. Therefore, at least until the number of laryngologists has caught up with the number needed, we must be especially diligent about defining expected areas of basic knowledge.

Details of a recommended curriculum are beyond the scope of this paper. However, suggestions have been articulated, at least in preliminary form. One such document was developed by the Speech, Voice and Swallowing Committee of the American Academy of Otolaryngology, in a subcommittee chaired by Dr Steven Zeitels (personal communication). However, to date, no proposal has been accepted by the bodies that guide otolaryngology residency training.

The thoughts that follow constitute only a broad overview of my vision of minimum residency requirements with regard to substantive knowledge and clinical skill. They are offered not as definitive recommendations, but rather, to encourage dialog and an eventual consensus. This paper concentrates on voice because voice is the most advanced and complex division of laryngology at present, and in order to limit the length of this paper. A similar model should be applied to training in the management of swallowing disorders and selected speech and language disorders.

Basic Science. Comprehensive knowledge of relevant anatomy, physiology, and pathology is required for insightful diagnosis and expert treatment of laryngological patients. Every trainee should be familiar with the layered microanatomy of the vocal fold, the characteristics of its basement membrane, neuromuscular anatomy (including the latest concepts in fiber composition and subspecialization within given muscles), laryngeal aging (from embryo to death), and the nature and importance of supraglottic and infraglottic components of the vocal tract. Neurolaryngology has emerged as a new field, and is vitally important to the clinician. Just as neurotology has expanded our scope of training in otology, so must neurolaryngology in laryngology. Training should include elements of neuroanatomy and neurophysiology that seemed irrelevant until recently. Every graduate of a residency should also be familiar with the intricacies of voice physiology, including respiration, infraglottic power-source functions, the details of sound production at the level of the vocal folds, and the resonator functions of the supraglottic vocal tract. Understanding each component of the anatomy and physiology is not simply an academic exercise. Such knowledge allows the clinician to perform a "systems analysis" on the voice, determine which components are malfunctioning or misfunctioning, and establish diagnoses and treatment paradigms rationally.[28] Moreover, just as we expect our trainees to understand audiograms and how to interpret them, every trainee should be familiar with instrumentation for objective voice quantification and should be able to interpret data generated from voice laboratories. They should also be familiar with instruments for outcomes assessment in patients with voice disorders.[29]

Research. Research is an essential component of any postgraduate training program. Ultimately, at its best, research is the means by which we figure out how to improve the condition of patients whom we cannot help now. Each resident should receive training in research methodology and should have practical experience with basic and/or clinical research. Such training is essential not only to teaching incisive interpretation of the literature, but moreover, to developing the ability to formulate precise questions relevant to the practice of laryngology, and to design rational paths toward their answers. Good research training should help solidify the dissatisfaction all physicians feel about our limitations, and it should encourage a lifelong curiosity and an unwillingness to accept the limitations of our knowledge. Research should become a vital tool of daily practice through which we advance the boundaries of our specialty and enhance the care of our patients.

Diagnosis and Medical Management. Residents should master the details of the comprehensive, multisystem history required for patients with voice disorders, with special techniques for physical examination, and be familiar with the many other special considerations that must be taken into account when caring for voice professionals.[30-33] They should be able to recognize not only obvious laryngological problems such as benign and malignant vocal fold lesions, but also less obvious lesions and related disorders. Graduates of our residencies should be able to differentiate voice dysfunctions emanating from the infraglottic or supraglottic vocal tract, as well as laryngeal manifestations of systemic disease (reflux, thyroid disease, and many others). They should also gain experience in performing and interpreting strobovideolaryngoscopy and be familiar with the application of other diagnostic tests, including acoustic analysis, airflow assessment, laryngeal electromyography, and others. In addition, otolaryngology residents should receive specific training in the principles and practice of voice therapy, and they should spend at least some time observing therapy performed by an expert speech-language pathologist. Sufficient knowledge should be imparted to allow any laryngologist to determine whether a specific speech-language pathologist is providing his or her patient with appropriate, safe, and beneficial therapy. Physicians who refer their patients to speech-language pathologists cannot fulfill this basic obligation unless they have a reasonable understanding of the indications for referral, of the techniques utilized by modern voice therapists, and of the expected duration and progress of therapy. Ideally, residents should also be given an opportunity to work with a multidisciplinary voice team. It is also essential, of course, for trainees to be familiar with medical treatment of voice disorders, including the vocal consequences of various medications prescribed by otolaryngologists and other physicians (iatrogenic dysphonias).

In addition, we must prepare our trainees for "special situations" — for example, complex problems such as laryngeal trauma, vocal fold scarring, and arytenoid dislocations that often require special expertise and/or rapid diagnosis and management. High-performance professional voice users also pose special challenges, obligations, and risks with which any practicing otolaryngologist should be familiar in order to avoid well-meaning but potentially costly treatment errors.

Surgery. Otolaryngology boasts an excellent tradition of teaching surgical skills. Prior to performing neck surgery on humans, residents have been instructed on the anatomy and physiology of neck structures and pathophysiology, and they have performed neck dissections on cadavers. They have also generally observed or assisted in numerous similar surgical procedures. The training tradition is even more consistent and rigorous for surgery of the temporal bone. Temporal bone laboratory dissection with professorial instruction and supervision is required in most programs before residents are entrusted with surgical care of human ears. Unfortunately, the same systematic approach to teaching of surgical skills is often not applied to laryngeal surgery in most institutions. Laryngeal endoscopy, microscopic voice surgery, and phonosurgery (including framework surgery) are amenable to a similar thorough and systematic approach to training. In addition to instruction in anatomy and physiology of phonation, examination and quantification of voice function, outcomes assessment, alternatives to surgery, and timing of surgery before operating, the resident should have a thorough knowledge of surgical instrumentation (traditional and laser). Practice under supervision in laryngeal dissection laboratories (similar to temporal bone laboratories) and periods of observation in the operating room should precede resident surgery on human larynges. Attention to surgical technique and ergonomics is essential, because of the technical difficulties involved in maintaining perfect control of the tips of long laryngeal instruments. Indeed, laryngeal microsurgery may well be approached conceptually as ear surgery with longer instruments. Resident surgery on patients should be supervised closely and should follow a planned progression from simple to complex cases; and it should be recognized that microdissection of the vibratory margin of the vocal fold may be more challenging technically than some seemingly "larger" cases such as laryngectomy. Results from residents' surgery should be comparable to those achieved by professorial faculty. Training should also include criteria for determining which cases should not be performed by the inexperienced or occasional laryngeal surgeon, and guidelines for referral to tertiary care laryngologists.

Special Considerations. In addition to providing training in facts and skills, attention must be paid to the art of patient care. This is extremely important in the management of all patients, and acutely important when caring for high-performance voice professionals.

Residents in otolaryngology, and fellows in laryngology and voice, should also be imbued with curiosity about the many questions that remain unanswered. They should receive historical information about the development of our field, and exposure to voice professionals in other disciplines such as speech-language pathologists, singing teachers, and arts-medicine specialists in other fields.

FELLOWSHIP TRAINING

Fellowship programs in laryngology have proliferated recently in response to clinical and academic demand, but they have actually existed for many years. For example, Dr Hans von Leden and the late Dr Wilbur James Gould have trained fellows since the 1950s and 1960s, among them such distinguished individuals as Drs Minoru Hirano and Nobuhiko Isshiki. This author began providing such training in 1981; and since that time, fellows have included not only laryngologists, but also speech-language pathologists and singing teachers. If our specialty is to provide enough practitioners to meet the need for high-caliber laryngology and voice care teams, then we must participate in advanced training for physicians and for professionals in other disciplines who will constitute the interdisciplinary teams of the future.

While no formal guidelines for fellowship training have been accepted or implemented to date, they may be anticipated in the near future. A draft document has been developed by Dr Robert Ossoff, this author, and other members of The Ad Hoc Committee on Laryngology Fellowship of the ALA. The need for quality control and some standardization in minimum experience in fellowship programs will hopefully lead to further action on this document or a similar set of guidelines in the near future.

CREATIVE THOUGHT

Interdisciplinary opportunities for creativity in medicine still offer the potential for excitement, joy, and innovation in daily practice.[34] The current advances in laryngology were inspired by interest in the problems of professional voice users, particularly opera singers; but this trend in laryngology was not isolated. Modern voice medicine is but one component of a larger field of arts medicine that offers similar interdisciplinary team care for pianists, string players, dancers, wind instrumentalists, and others.[35] The arts-medicine aspects of laryngology are exciting for at least 2 reasons that should be addressed in any training program. First, performers and artists place critical demands on their bodies, and they do not have the usual tolerance for incomplete cures. An injured finger that returns to 98% normal function may be adequate even for a microsurgeon, but it is not adequate for a premier pianist. That extra 2% separates the famous artist from those who have not quite reached the "top." Arts-medicine patients force us to redefine "normal" much more narrowly, and they challenge our abilities to recognize, quantify, and restore physiological perfection. Much of the fun and many of the ideas that have helped advance laryngology and voice have come from close interactions with such patients, as well as with speech-language pathologists, voice scientists, singing teachers, acting teachers, and other colleagues, all of whom provide insights useful in clinical practice. Second, arts medicine provides physicians with an opportunity to work closely with professionals and educators in the arts and humanities. The arts and medicine are inherently similar in many ways. However, through our educational process, physicians too often lose sight of the importance of the arts to our practices, and there is a movement in medical education to correct this deficiency. Arts medicine offers the clinician a chance to work and think with colleagues in related fields, such as artists and performers, and to find new solutions to complex problems. It also may afford us with the inspiration and opportunity to study one of the arts. In our quest to master the science and art of healing, we can learn much from our colleagues in the arts and humanities that will help our insight, sensitivity, and ability to empathize. Such interactions also help keep us from being trapped intellectually by existing paradigms and allow us to approach questions with a broader vision, creating new solutions to problems that seem insurmountable within the compartmentalized framework of our traditional training, and even allow us to create new fields of medicine.

CONCLUSION

Laryngologists of the future will be ideally positioned to thrive as sophisticated diagnosticians, surgeons, and scientists; but they will also have exceptional opportunities to remain "physicians" in the truest and broadest sense. It is incumbent upon us to offer training environments that will not merely provide skills, but will also kindle and nurture their curiosity, creativity, and broader vision. In these days of economic and legal crises, medicine has precious few reminders of the reasons why most of us were inspired to become doctors. For the last 2 decades, modern laryngology has been built on such enthusiasm, and practicing it has been a daily joy. We must ensure that the next generation will not have to settle for anything less. If we are successful, 20 years from now, our practice will look as crude as Elsberg's methods seem to us now; and we will have accomplished our mission as educators and left behind training centers filled not only with information, but also with inspiration and imagination.

REFERENCES

1. Elsberg L. Presidential Address to the First Meeting of the American Laryngological Association. Trans Am Laryngol Assoc 1879;1:33-90.
2. Elsberg L. Presidential Address to the Second Meeting of the American Laryngological Association. Trans Am Laryngol Assoc 1880;2:3-11.
3. Knight F. Presidential Address to the Fourth Meeting of the American Laryngological Association. Trans Am Laryngol Assoc 1882;4:2-11.
4. Wilders JSS. On the teaching of laryngology. Br Med J 1890;2:376-7.
5. Swain HL. Laryngology and its place in medical education: Presidential Address to the Twenty-Third Meeting of the American Laryngological Association. Trans Am Laryngol Assoc 1901;23:1-17.
6. Knight CH. The teaching of laryngology, then and now. Laryngoscope 1906;160:840-3.
7. Harris TJ. The training of the specialist. Ann Otol Rhinol Laryngol 1913;22:475-81.
8. Skillern RH. Post-graduate work in laryngology. JAMA 1921;77:1145-6.
9. Milligan W. The rise and progress of laryngology: its relation to general medicine and its position in the medical curriculum. Br Med J 1922;1:547-51.
10. Dean LW. The teaching of undergraduate laryngology. Laryngoscope 1925;35:735-41.
11. Layton TB. The aims and methods of teaching laryngology. J Laryngol Otol 1940;55:495-502.
12. Alford BR. The age of the medical education revolution. Laryngoscope 1966;106:801-4.
13. Bailey BJ. Laryngology education at the turn of the century. Laryngoscope 1966;106:797-800.
14. Ridpath RF. Diseases of the larynx. In: Jackson C, Coates GM, eds. The nose, throat, and ear and their diseases. Philadelphia, Pa: WB Saunders, 1929:736-44.
15. Shahrokh DK, Devine KD. A teaching device for residents in laryngology. Arch Otolaryngol 1961;74:234-5.
16. Garcia M. Observations on the human voice. Proc R Soc Lond 1855;7:397-410.
17. Zeitels SM. Premalignant epithelium and microinvasive cancer of the vocal fold: the evolution of phonomicrosurgical management. Laryngoscope 1995;105(suppl 67).
18. LeJeune FE. Motion picture study of laryngeal lesions. Surg Gynecol Obstet 1936;62:492-5.
19. Moore P, von Leden H. Television in otolaryngology and other specialties. A new teaching device. JAMA 1959;169:1976-80.
20. von Leden H. Laryngeal physiology: cinematographic observations. J Laryngol Otol 1960;74:705-12.
21. Moore GP, White FD, von Leden H. Ultra high speed photography in laryngeal physiology. J Speech Hear Disord 1962;27:162-71.
22. von Leden H, Le Cover M, Ringel RL, Isshiki N. Improvements in laryngeal cinematography. Arch Otolaryngol 1966;83:482-7.
23. Tardy ME. Microscopic laryngology: teaching techniques. Laryngoscope 1972;82:1315-22.
24. Hirano M. Structure and vibratory pattern of the vocal folds. In: Sawashima N, Cooper FS, eds. Dynamic aspects of speech production. Tokyo, Japan: University of Tokyo Press, 1977:13-24.
25. Sataloff RT. The human voice. Sci Am 1992;267:108-15.
26. Sataloff RT. Professional voice: the science and art of clinical care. 2nd ed. San Diego, Calif: Singular Publishing Group, 1997.
27. National strategic research plan of the National Institute on Deafness and Other Communication Disorders, 1991, 1992, 1993. NIH publication #95-3711. Bethesda, Md: US Department of Health and Human Services, 1995:270.
28. Sataloff RT. Rational thought: the impact of voice science upon voice care. J Voice 1995;9:215-34.
29. Benninger MS, Gardner GM, Jacobson BH, Grywalski C. New dimensions in measuring voice treatment outcomes. In: Sataloff RT. Professional voice: the science and art of clinical care. 2nd ed. San Diego, Calif: Singular Publishing Group, 1997:789-94.
30. Sataloff RT. Patient history. In: Sataloff RT. Professional voice: the science and art of clinical care. 2nd ed. San Diego, Calif: Singular Publishing Group, 1997:193-201.
31. Raphael BN. Special considerations relating to members of the acting profession. In: Sataloff RT. Professional voice: the science and art of clinical care. 2nd ed. San Diego, Calif: Singular Publishing Group, 1997:203-5.
32. Sataloff RT. Physical examination. In: Sataloff RT. Professional voice: the science and art of clinical care. 2nd ed. San Diego, Calif: Singular Publishing Group, 1997:207-13.
33. Sataloff RT, Heuer R, Emerich KA, Baroody MM, Rulnick RK, Hawkshaw MJ. The clinical voice laboratory. In: Sataloff RT. Professional voice: the science and art of clinical care. 2nd ed. San Diego, Calif: Singular Publishing Group, 1997: 215-43.
34. Sataloff RT. Interdisciplinary opportunities for creativity in medicine. Ear Nose Throat J 1998;77:530-3.
35. Sataloff RT, Brandfonbrener A, Lederman R, eds. Performing arts medicine. 2nd ed. San Diego, Calif: Singular Publishing Group, 1998.

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INHIBITION OF SUBGLOTTIC STENOSIS WITH MITOMYCIN-C IN THE CANINE MODEL

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The objective of this randomized, prospective study was to study the efficacy of topical mitomycin-C in the inhibition of subglottic stenosis in a canine model. Subglottic stenosis was elicited with the carbon dioxide laser in 10 mongrel dogs. Radial incision and serial dilation of the subglottic airway were then carried out. The animals were randomized to receive a topical solution of 1% mitomycin-C to the dilated area for a 5-minute duration or no further treatment. Weekly direct microlaryngoscopy and photodocumentation were performed during the 6-week study. Airway distress developed in 4 of the 5 control dogs, requiring early sacrifice, while all treatment group animals survived the duration of the study ($p \leq .006$). Morphometric analysis of the subglottic photographs confirmed a greater than 100% increase in the percentage of relative airway at sacrifice in the treatment group ($p \leq .049$). A statistically significant ($p \leq .015$) decrease in collagen formation in the subglottic scar of dogs treated with topical mitomycin-C was documented. Mitomycin-C favorably altered the clinical progression of subglottic stenosis, improved quantified airway patency, and reduced the amount of subglottic collagen formation in the canine model.

KEY WORDS — canine, mitomycin-C, subglottic stenosis.

INTRODUCTION

Acquired subglottic stenosis is a debilitating condition and a challenging disease process for the otolaryngologist to treat. Often, patients require multiple endoscopic or open reconstructive procedures, and they may ultimately become tracheostomy-dependent. In an effort to better understand the pathophysiology of this disease and to test treatment options, animal models have been developed.

Borowiecki and Croft[1] first described a canine model for subglottic stenosis in 1977. Many studies since that time have attempted to identify adjunctive agents to traditional therapy in maintaining subglottic patency. The role of antibiotics, steroids, and lathyrogenic agents such as penicillamine and *N*-acetyl-L-cysteine has been evaluated, but none has significantly impacted clinical practice.[2-4] Charous et al[5] reported on the use of the carbon dioxide (CO_2) laser to create subglottic injury in the dog and further documented the clinical and histologic progression of stenosis in this model. They found that extensive cartilaginous injury was required for stenosis to form, supporting the findings of previous studies[6,7] in which the cartilage was damaged with prolonged endotracheal intubation. Charous et al found that stenosis would develop in a time period from 2 to 3 weeks postinjury. On average, the dogs would restenose 10 days after the subglottic scar was excised with the CO_2 laser. This study provided a predictable model to test agents for the inhibition of scar formation in the subglottis.

Mitomycin-C is an antibiotic isolated from the broth of the bacterium *Streptomyces caespitosus*. It has been found to have antitumor activity and has been used in multidrug regimens for disseminated adenocarcinoma, as well as transitional cell tumors of the genitourinary tract. The compound selectively inhibits DNA synthesis through mitomycin-C–induced cross-linking. This primarily occurs at guanine and cytosine, and thus, concentrations of these nucleic acids correlate to efficacy. At high drug concentrations, RNA and protein synthesis are suppressed. These properties have led to its use as an agent for reducing scar formation.

The objective of this randomized, prospective study was to evaluate the efficacy of topical mitomycin-C in the inhibition of subglottic stenosis by means of a canine model. The clinical course of restenosis and airway distress would be monitored. Additionally, airway patency would be photodocumented and

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From the Department of Otolaryngology, Vanderbilt Bill Wilkerson Center for Otolaryngology and Communication Sciences, Nashville, Tennessee. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Vanderbilt University.

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then analyzed quantitatively. Histologic analysis of scar formation in the 2 groups would be carried out to describe the effects of mitomycin-C on the subglottic scar and to compare the treatment group with the control group.

MATERIALS AND METHODS

Ten adult mongrel dogs weighing between 15 and 20 kg were utilized for the study. The animal research was approved and conducted according to our Institutional Animal Care and Use Committee (IACUC). Anesthesia was begun with a mixture of butorphanol tartrate (0.05 mg/kg) and atropine sulfate (0.05 mg/kg) given intramuscularly for premedication. Induction was then carried out with tiletamine hydrochloride (4 to 6 mg/kg) and xylazine hydrochloride (0.275 mg/kg) given intramuscularly. Intravenous maintenance doses of tiletamine hydrochloride (2 to 4 mg/kg) and xylazine hydrochloride (0.2 mg/kg) were given as needed.

Direct microlaryngoscopy was performed with a suspension laryngoscope modified for use in the dog. An operating microscope with a 400-mm–focal length lens was then moved into position. A CO_2 laser (Sharplan Lasers Inc, Allendale, NJ) was attached to a 250-µm–spot size micromanipulator. Circumferential injuries at the level of the cricoid and inferior to the second tracheal ring were created with 6 W of power in the continuous mode. The depth of injury was through the level of the perichondrium and into tracheal cartilage, as characterized by the typical "welder's flash."

Weekly direct microlaryngoscopy was performed to assess the progression of stenosis. Photodocumentation was carried out with a 0° Hopkins (Karl Storz Inc, Tuttlingen, Germany) rigid telescope with a camera lens attachment. All camera settings on the Nikon N6006 (Nikon Corp, Tokyo, Japan) camera body were standardized and maintained between dogs and operative procedures. When stenosis was clinically assessed to reach at least a 50% reduction in available airway lumen, the dogs were treated with CO_2 laser radial incisions in 4 quadrants and then with serial dilation by using rigid bronchoscopes in the method of Ossoff et al.[8] The bronchoscopes ranged in size from 3.5 to 9 mm. Dogs were then randomized to receive a topical application of 1% (10 mg/mL) mitomycin-C (Bedford Labs, Bedford, Ohio) or no further treatment. The solution was created by suspending the drug in 1 mL of sterile water. Drug delivery was via a soaked cotton pledget directly applied to the operative site for a duration of 5 minutes.

The clinical course of restenosis and airway distress was monitored daily. Weekly direct microlaryngoscopy and photodocumentation continued after treatment. Dogs were painlessly sacrificed if clinical signs of airway distress developed and direct microlaryngoscopy confirmed a significant reduction in airway lumen. The study duration was 6 weeks from the time of treatment, and all living animals were painlessly sacrificed at that time. Sacrifice was carried out with an intracardiac injection of pentobarbital sodium after the induction of anesthesia.

Histologic Procedure. The larynx and proximal trachea were harvested from each dog and immediately placed in 10% formalin. After an appropriate time for fixing, the subglottis and proximal trachea of each specimen were cut transversely into approximately 3-mm sections (5 to 8 sections per animal). Specimens were dehydrated in a graded series of ethanols, embedded in paraffin wax, and cut into 5-µm sections by standard techniques. Tissue was mounted on slides and stained with hematoxylin and eosin or Masson's trichrome stain.

Microscopic Analysis of Specimens. Slides were viewed with an Olympus Vanox AH-2 light microscope (Lake Success, NY) under 40× magnification. Each section of the specimen was examined for degree of epithelialization, scar as assessed by collagen formation, and inflammatory response. For computer analysis, images were digitally photographed with a ProgRes 3008 camera (Kowtron Elektronik, Eching, Germany) and imported into Adobe Photoshop 4.0 (Mountain View, Calif). Images were captured at 1,996 × 1,450 pixels in 24-bit color. If the area of interest extended beyond a single camera field, multiple images were captured and then montaged together with Photoshop.

Computer Image Analysis. Computer imaging (morphometric analysis) was used to determine the percentage of collagen present at the point of maximal airway stenosis for each animal. This was determined by visually inspecting the histologic slides for the point of thickest scarring and by using light microscopy to verify the histologic appearance of scar tissue. On the Masson's trichrome–stained slides, a digital image at the point of maximal stenosis was captured for each specimen. To measure the amount of collagen present, a reference box 1.0 cm × 500 µm was defined. The box size was selected to be long enough to include the full thickness of scar formation for the maximally stenosed sample, and wide enough to allow a representative sample of the tissue. With Photoshop, the box was superimposed over each image, beginning at the edge of the cartilage and extending into the lumen (Fig 1). The range of blue pixels representing the stained collagen fibers was defined for each image by the investigator. These

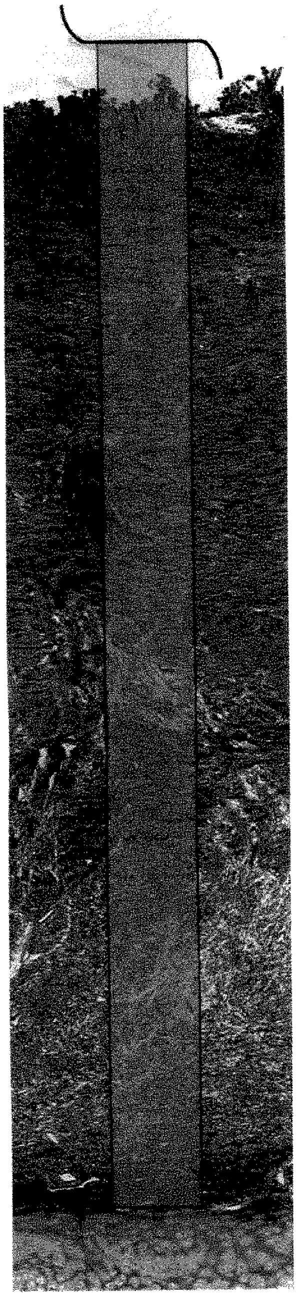

Fig 1. Masson's trichrome stain of control animal subglottis at 40× magnification. Standard reference box was created with Photoshop and oriented from cartilage to lumen.

values were then normalized by the maximal value of all the samples measured to produce the relative collagen formation score (in percent) for each animal. This technique was used rather than simply measuring the distance of stenosis because it would be less affected by mounting variation or artifact. An unpaired Student's t-test was performed with StatView (Abacus Concepts Inc, Berkeley, Calif) to determine if there was a difference in the collagen formation at the point of maximal stenosis between the control and treatment groups.

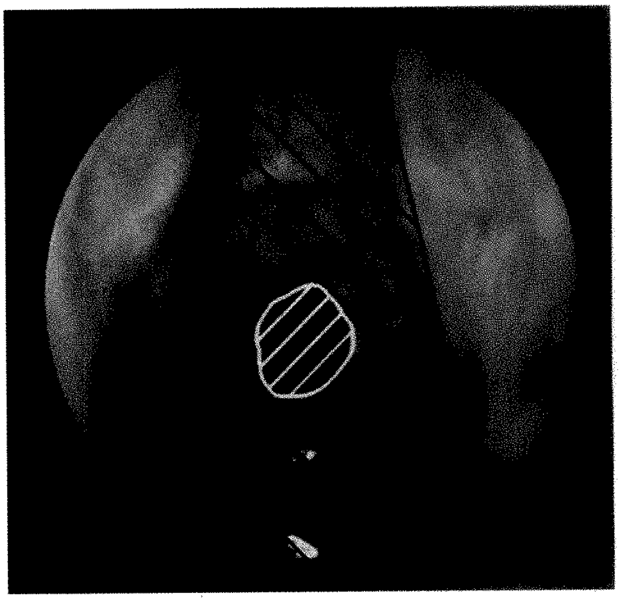

Fig 2. Sample subglottic photograph of animal with subglottic stenosis. Narrowest point in airway lumen is shown with white shading superimposed. Lumen size at most inferior aspect of true vocal folds, used as fixed reference, is shown with black shading superimposed. Ratio of white to black is defined as percentage relative airway as explained in text.

Photodocumentation Analysis. Morphometric analysis with Photoshop was also used to assess the clinical photographs documenting the progression of stenosis. Slide photographs taken at the time of initial treatment and at the time of sacrifice were analyzed in a similar fashion. After the 35-mm slides were scanned at 300 dpi resolution, the number of pixels at the narrowest point in the airway lumen was counted (see white shading on Fig 2). We then had to normalize the total pixels measured in the narrowest points of the airway lumen to a fixed reference in the subglottis. The lumen size at the most inferior aspect of the true vocal folds was chosen for the fixed reference. So, the number of pixels within the most inferior aspect of the true vocal folds was counted (see black shading in Fig 2). The number of pixels measured at the narrowest point was then divided by the number of pixels from the fixed reference. To avoid confusion, we term this normalized cross section a "percentage relative airway." This use of the relative airway allowed for standardization between photographs, decreasing the interanimal variations. It also served to negate any difference in magnification between individual photographs and to compensate for any size difference between animals. The percentages of relative airway of the 2 groups at treatment and at the time of sacrifice could then be compared. Data were also analyzed with an unpaired Student's t-test and StatView. We note that the morphometrically determined percentage relative airway

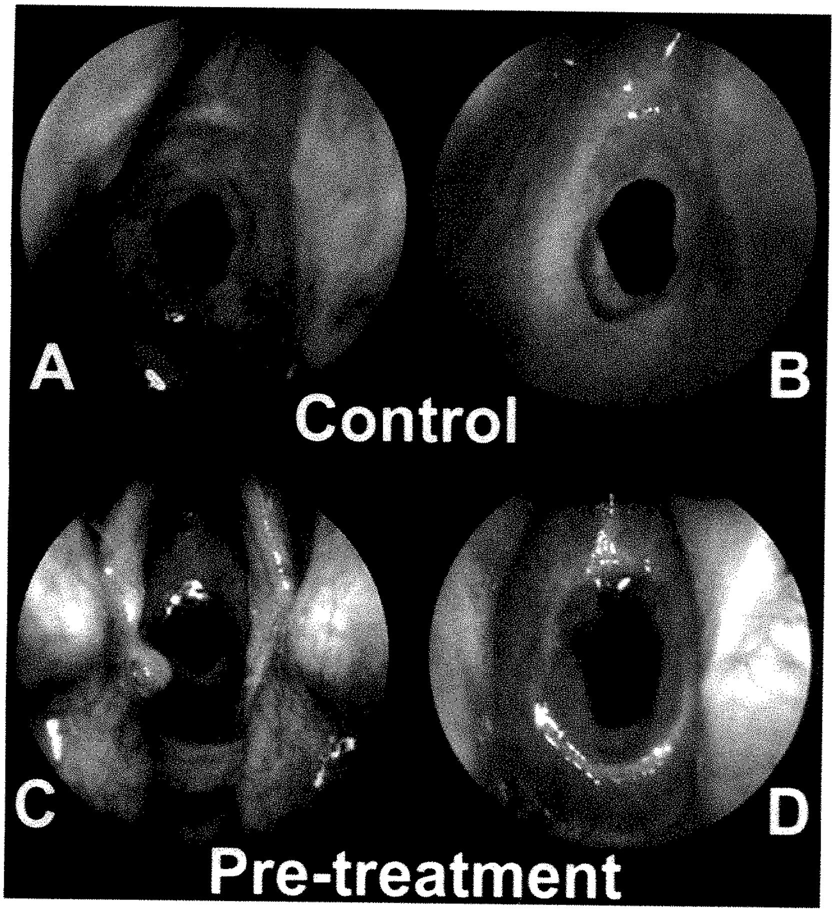

Fig 3. Sample subglottic photographs. A,B) Two control animals prior to treatment with radial incision and serial dilation. C,D) Two treatment animals prior to radial incision and serial dilation with subsequent application of topical mitomycin-C.

yields a value that is typically much smaller than one would subjectively assess as a percentage airway opening.

RESULTS

Clinical Course. All dogs in the mitomycin-C treatment group survived the full 6-week duration of the study. None developed respiratory distress. There were no complications of topical mitomycin-C use noted in these animals. Four of the 5 dogs in the control group developed stridor and required sacrifice prior to study termination. Clinical restenosis was documented by direct microlaryngoscopy and photodocumentation prior to sacrifice. The 1 dog in the control group that did not progress to restenosis lived for the duration. The other dogs succumbed at 8, 9, 13, and 21 days. Statistically significant improvements in the clinical course of stenosis and airway distress were noted in the treatment group as measured in days of survival ($p \leq .006$).

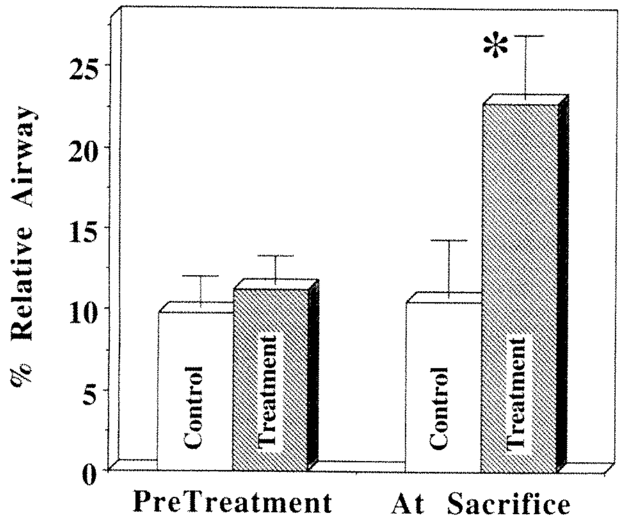

Fig 4. Graph of mean percentage relative airway values in both groups. Comparable degrees of stenosis are noted in both groups before treatment with radial incision and serial dilation. Significant difference is noted between groups at time of sacrifice. Asterisk — $p \leq .049$.

Clinical Photographs. The first goal in analysis of the subglottic photographs was to confirm the clinical observation that there was a significant reduction in the subglottic lumen ($\geq 50\%$ reduction by visual inspection) before radial incision and serial dilation were performed. Figure 3A,B includes representative photographs of 2 control dogs just prior to radial incision and serial dilation. Tight, circumferential stenoses are evident, and these correlated with the clinical presentation of increasing stridor. Similar analysis of photographs in the mitomycin-C treatment group also confirmed the high grade of steno-

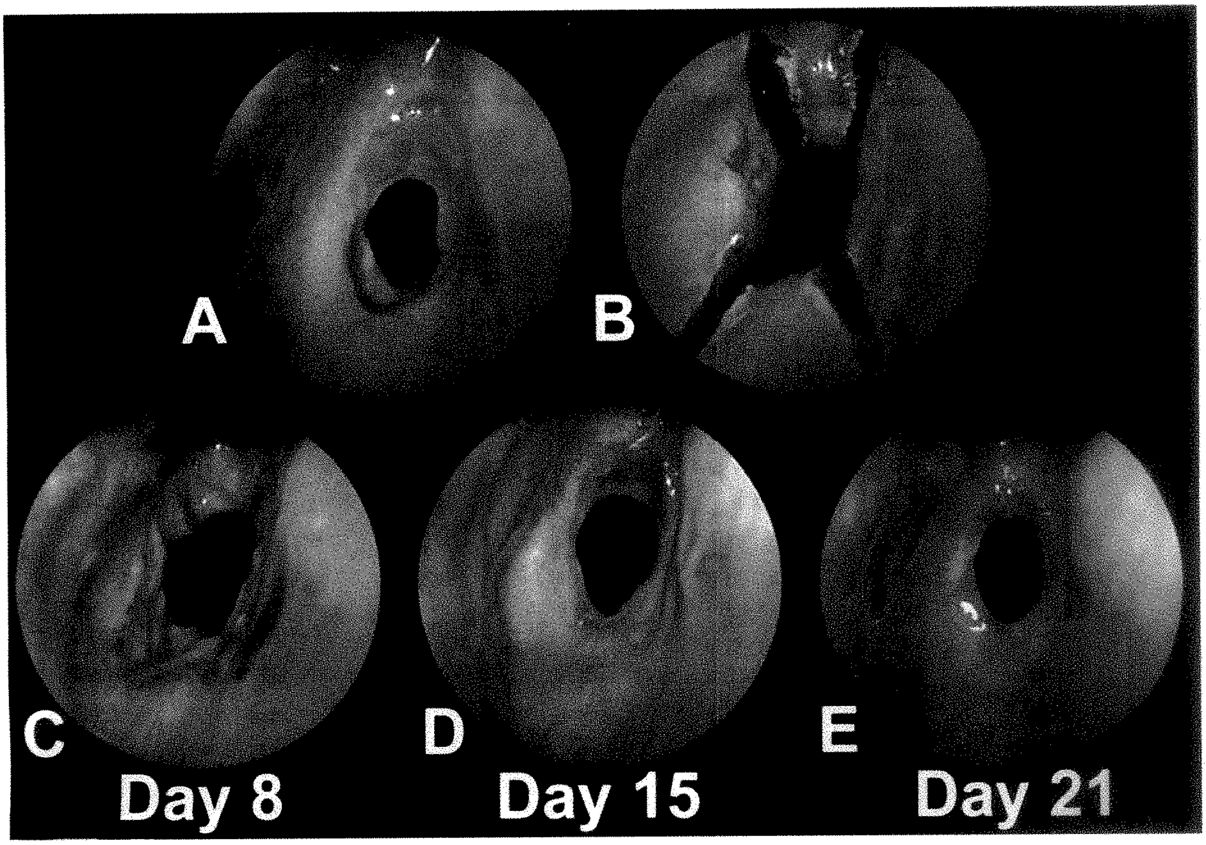

Fig 5. Documented clinical course of control animal. A) Tight, circumferential stenosis with clinical airway reduced by ≥50% and stridor noted. B) Radial incisions are performed (photograph predilation). C) Day 8; development of granulation and circumferential restenosis. D) Day 15; rapid progression of stenosis. E) Day 21; tight stenosis and respiratory distress noted.

sis that was observed at the time of treatment, and stridor was also noted (Fig 3C,D). It was then desirable to draw comparisons between the calculated percentage relative airway in the control group and that in the treatment group at the time of radial incision. This would serve to confirm that both groups were indeed being treated at a similar degree of stenosis. The mean percentage relative airway calculated for the control group at treatment was 9.8% ± 1.9% (Fig 4). The mean percentage relative airway calculated for the mitomycin-C group at the time of treatment was 11.2% ± 1.8%. The means of each group fell within the standard errors of one another and further supported the clinical impression noted upon direct microlaryngoscopy.

Figure 5 documents the clinical course of 1 of the control animals. The degree of stenosis at the time of radial incision and serial dilation is shown. By day 8, circumferential granulation and stenosis are developing. At day 15, this has progressed to a more mature scar, and by day 21, tight stenosis and respiratory distress are noted. Figure 6 shows the clinical course of 1 of the mitomycin-C group animals. The drug is applied topically to the site after dilation. At day 15, some circumferential narrowing has developed, but at 27 days, this has not progressed. Forty days posttreatment, the dog has a widely patent airway and no sign of distress.

The next objective was to analyze the photographs of the 2 groups at the time of sacrifice. The ratio of narrowest airway lumen to maximal relative airway measured in pixels in the control group appeared smaller than that of the treatment group. This was confirmed by comparing the calculated percentage relative airway values between the 2 groups (Fig 4). The mean percentage relative airway calculated for the control group at sacrifice was 10.5% ± 3.6%. It is notable that this mean for the control group returned to within 0.7% of the pre–radial incision and serial dilation values. The mean percentage relative airway calculated for the mitomycin-C group at the time of sacrifice was 22.8% ± 3.9%. This greater than 100% difference in calculated percentage relative airway between the mitomycin-C group and the control group was statistically significant ($p \leq .049$).

Histologic Examination. Evaluation of the histologic sections taken from the subglottis and proximal trachea confirmed the presence of subglottic stenosis (Fig 7). Characteristic fine, irregular collagen

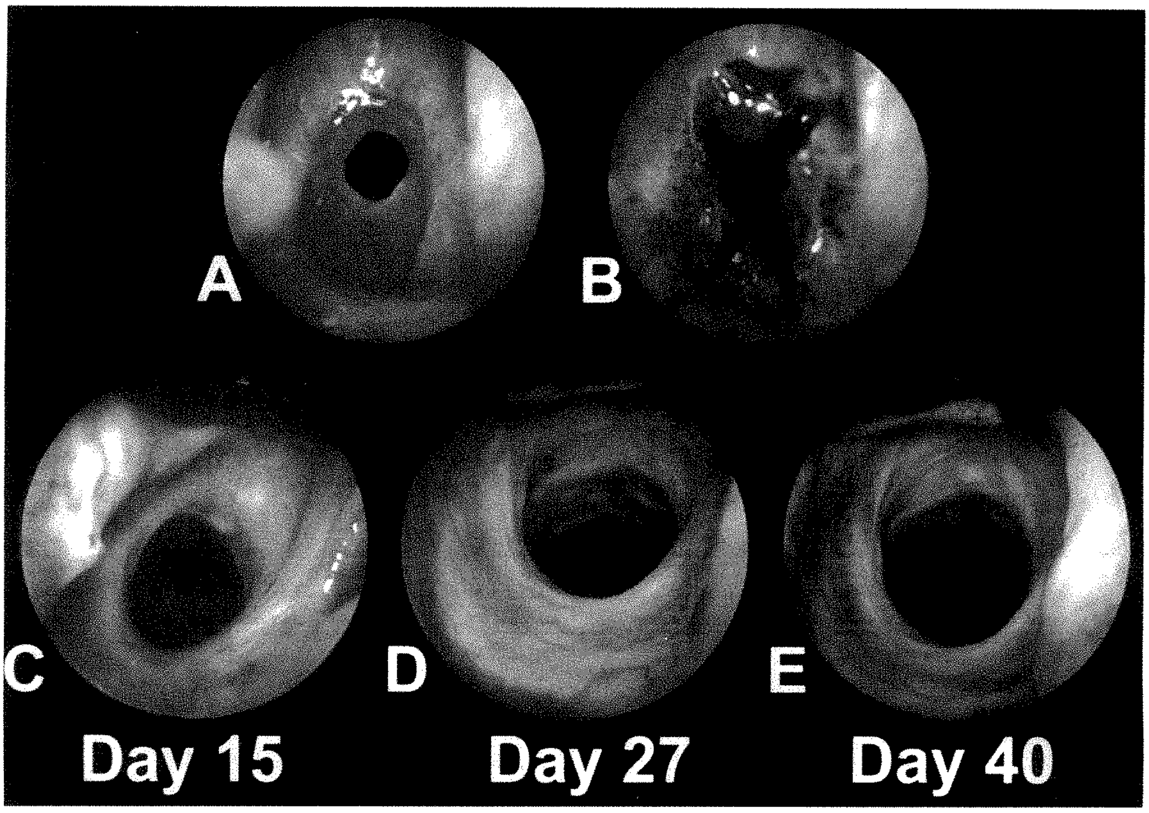

Fig 6. Documented clinical course of treatment animal. A) Tight, circumferential stenosis with clinical airway reduced by ≥50% and stridor noted. B) Radial incisions are performed with subsequent application of topical mitomycin-C (photograph predilation). C) Day 15; small amount of restenosis present. D) Day 27; little progression of stenosis. E) Day 40; widely patent airway with no evidence of respiratory distress near end of study.

fibers were seen throughout the submucosa. Additionally, extensive neovascularization was present. Re-epithelialization with respiratory mucosa was noted in all specimens in both groups. Masson's trichrome–stained slides revealed the blue-staining collagen that was then quantified. The relative percentage of collagen formation for the 2 groups was determined by using the previously described technique of computer image analysis. The mean relative collagen percentage for the control group was 73.6% ± 13.4%. The mean relative collagen percentage for the mitomycin-C group was 28.9% ± 5.7%. This represented a statistically significant ($p \leq .015$) decrease in collagen formation in the subglottic scar of dogs treated with topical mitomycin-C.

DISCUSSION

The properties of mitomycin-C as an inhibitor of fibroblast proliferation and vascular ingrowth in tissues have led to its clinical use in ophthalmologic surgery. Kunitomo and Mori,[9] in 1963, first introduced its topical use in the eye after pterygium surgery. In 1983, Chen[10] reported using mitomycin-C as adjunctive therapy in trabeculectomy surgery for glaucoma. He found that it reduced scarring at the scleral flap with a 100% success rate at the relatively low dose of 0.02%. A double-blinded, prospective study in 1988[11] helped popularize its use in North America by showing a decrease in the pterygium recurrence rate from 88.9% to 2.3% when compared to placebo. The use of mitomycin-C topically on respiratory epithelium, specifically on nasal mucosa, was first explored as adjunctive therapy in dacryocystorhinostomy.[12] A recent study by Ingrams et al[13] found it to be efficacious in maintaining patent maxillary antrostomy sites in a rabbit model. Electron microscopy revealed that after an initial period of ciliary loss, no permanent damage to the mucosa was noted and there was a return of normal ciliary function within 1 week.

In systemic use of mitomycin-C as cancer chemotherapy, myelosuppression has been the primary documented toxicity. With topical human use, only local ocular findings have been reported. In pterygium surgery, scleral and corneal ulceration, scleromalacia, symblepharon, calcification, iritis, corneal perforation, mature cataract formation, and ocular hypotonia have been documented.[14] The use of lower doses and exposure times has resulted in a dramatic reduction in these complications.[15-17]

In this study using topical mitomycin-C in the ca-

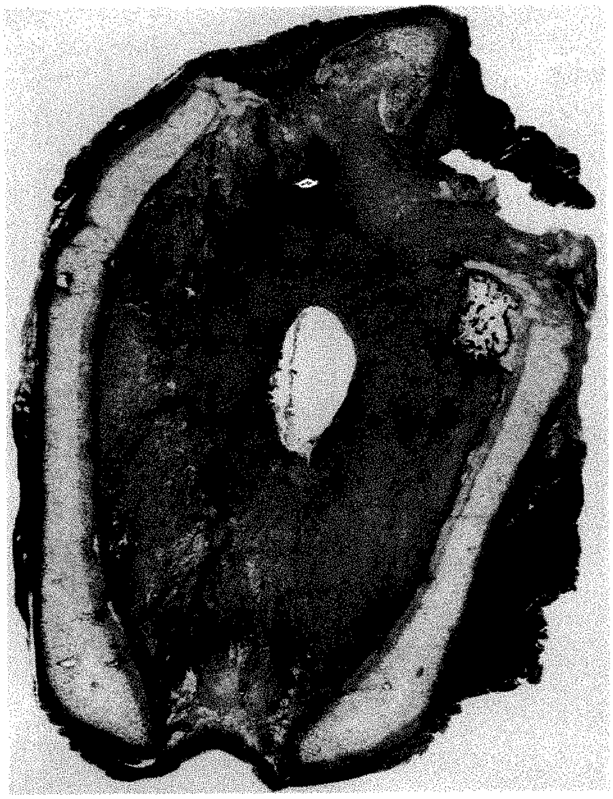

Fig 7. Masson's trichrome stain of control animal subglottis at low power. Whole organ section reveals characteristic fine, irregular collagen fibers throughout submucosa with extensive neovascularization present. Re-epithelialization with respiratory mucosa was noted in all specimens in both groups.

nine subglottis, a number of positive outcomes support its role as a possible adjunct to current therapy in treating patients with subglottic stenosis. First, the model of CO2 laser–induced subglottic stenosis reported by Charous et al[5] was found to be reproducible and effective for studying the pathophysiology of this disease process and a new agent for scar inhibition. Animals in the mitomycin-C group had a significant improvement in the clinical progression of subglottic stenosis relative to the control group. The clinical responses that were noted in daily evaluation and weekly direct microlaryngoscopy were supported by the photographic analysis. It is important to reiterate that the seemingly low percentage relative airway values in both groups highlight the fact that this calculated number is relevant mostly as a comparative tool between the 2 groups. We feel the actual clinically evaluated percentage available airway would have been reported as much higher by most surgeons, as can be seen in the Figure depicting a treatment animal at the time of sacrifice (Fig 6). Using pixels, a fixed reference point, and a ratio served to standardize the findings between photographs and dogs, allowing comparisons to be drawn. The greater than 100% increase in mean percentage relative airway for the treatment group should be evaluated in the context of the improved clinical course, lack of airway distress, and clinically larger airway lumens seen in these dogs.

There were no complications of mitomycin-C use noted in these dogs. The 1% concentration chosen for application was 10-fold higher than that in the recent publication on using mitomycin-C in the rabbit sinus model.[13] Our goal in this first reported use in the canine subglottis was to document its effects clinically, quantitatively, and histologically. Improvements in all these areas were noted, with no untoward effects at this higher concentration. Although there were no observable toxic effects, some degree of systemic absorption likely occurs through the mucosa at the area of treatment, and possibly a small amount distally into the trachea. Titration to dose-response levels and assessment of toxicity will need to be evaluated prior to application in human subjects. However, the side effects of systemic absorption are well documented and could be followed as with cancer chemotherapy patients.[18]

CONCLUSION

The clinical course of dogs treated once topically with mitomycin-C was significantly improved as compared with controls. Significant airway distress developed in 4 of the 5 control dogs, requiring early sacrifice, while all treatment group animals survived the duration of the study ($p \le .006$). Clinical impressions were supported by morphometric analysis confirming a greater than 100% increase in the percentage relative airway at sacrifice in the treatment group ($p \le .049$). A statistically significant ($p \le .015$) decrease in collagen formation in the subglottic scar of dogs treated with topical mitomycin-C was documented. Mitomycin-C favorably altered the clinical progression of subglottic stenosis, improved quantified airway patency, and reduced the amount of subglottic collagen formation in the canine model.

REFERENCES

1. Borowiecki B, Croft CB. Experimental animal model of subglottic stenosis. Ann Otol Rhinol Laryngol 1977;86:835-40.

2. Supance JS. Antibiotics and steroids in the treatment of acquired subglottic stenosis. A canine model. Ann Otol Rhinol Laryngol 1983;92:377-82.

3. Koufman JA, Branch ME, Ryu JH. A comparison of the carbon dioxide and neodymium:YAG lasers in a canine model of acquired subglottic stenosis. J Otolaryngol 1988;17:223-6.

4. Livingston GL, Schild JA. Lathyrogenic agents as therapy for subglottic stenosis — a pilot study. Otolaryngol Head Neck Surg 1987;97:446-51.

5. Charous SJ, Ossoff RH, Reinisch L, Davidson JM. Ca-

nine subglottic stenosis as a model for excessive fibrosis: a pilot histologic and immunohistochemical analysis. Wound Rep Reg 1996;4:444-53.

6. Marshak G, Doyle WJ, Bluestone CD. Canine model of subglottic stenosis secondary to prolonged endotracheal intubation. Laryngoscope 1982;92:805-9.

7. Supance JS, Reilly JS, Doyle WJ, Hubbard J, Bluestone CD. Acquired subglottic stenosis following prolonged endotracheal intubation. A canine model. Arch Otolaryngol 1982;108: 727-31.

8. Ossoff RH, Coleman JA, Courey MS, Duncavage J, Werkhaven JA, Reinisch L. A review of clinical applications of lasers in otolaryngology. Lasers Surg Med 1994;15:217-48.

9. Kunitomo N, Mori S. Studies on the pterygium. Part IV. A treatment of the pterygium by mitomycin-C instillation. Nippon Ganka Gakkai Zasshi 1963;67:601-7.

10. Chen CW. Enhanced intraocular pressure controlling effectiveness of trabeculectomy by local application of mitomycin-C. Trans Asia-Pacific Acad Ophthalmol 1983;9:172-7.

11. Singh G, Wilson MR, Foster CS. Mitomycin eye drops as treatment for pterygium. Ophthalmology 1988;95:813-21.

12. Kao SCS, Liao CL, Tseng JHS, Chen MS, Hou PK. Dacryocystorhinostomy with intraoperative mitomycin-C. Ophthalmology 1997;104:86-91.

13. Ingrams DR, Volk MS, Biesman BS, Pankratov MM, Shapshay SM. Sinus surgery: does mitomycin-C reduce stenosis? Laryngoscope 1998;108:883-6.

14. Rubinfeld RS, Pfister RR, Stein RM, et al. Serious complications of topical mitomycin-C after pterygium surgery. Ophthalmology 1992;99:1647-54.

15. Kitazawa Y, Suemori-Matsushita H, Yamamoto T, Kazuhide K. Low-dose and high-dose mitomycin trabeculectomy as an initial surgery in primary open-angle glaucoma. Ophthalmology 1993;100:1624-8.

16. Cheung JC, Wright MM, Murali S, Pederson JE. Intermediate-term outcome of variable dose mitomycin-C filtering surgery. Ophthalmology 1997;104:143-9.

17. Megevand GS, Salmon JF, Scholtz RP, Murray ADN. The effect of reducing exposure time of mitomycin-C in glaucoma filtering surgery. Ophthalmology 1995;102:84-90.

18. Ferguson MK. The effect of antineoplastic agents on wound healing. Surg Gynecol Obstet 1982;154:421-9.

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VIDEOLARYNGOSTROBOSCOPY FOLLOWING VERTICAL PARTIAL LARYNGECTOMY

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Phonation after partial laryngeal ablative surgery has not often been examined. Videolaryngostroboscopic recordings made after vertical partial laryngectomy (VPL) were retrospectively reviewed and correlated with patient historical and operative factors. Among VPL patients (n = 42), the most common site of vibration during phonation was the contralateral false vocal fold (17/42 patients or 40.5%), followed by the contralateral arytenoid mucosa (10/42 or 23.8%) and the contralateral true vocal fold (8/42 patients or 19.0%). There was no overall difference in vocal quality judgment with respect to site of vibration (ANOVA, p = .373). Vocal quality scores were similar with use of the pyriform mucosal flap versus other reconstructive methods (Student's *t*-test, p = .568). This study highlights the fact that reconstruction of a new vibratory source after VPL is important for voice production. Because VPL patients infrequently demonstrated true vocal fold vibration, alternative sites (ie, false vocal fold, arytenoid mucosa) must be considered as new phonatory sources after VPL.

KEY WORDS — vertical partial laryngectomy, videolaryngostroboscopy, voice.

INTRODUCTION

The unique anatomic structure of the human larynx has allowed for the development of a variety of oncologically sound vertical partial laryngectomy (VPL) procedures for early-stage carcinomas of the glottis.[1-6] However, the phonatory ability of the partially ablated and subsequently reconstructed larynx has not often been examined.[7,8] The question exists as to whether a variety of muscle or mucosal flaps used for neocord reconstruction can serve as viable options for postoperative voice production. Knowing the site of the laryngeal vibratory source after VPL would help the surgeon and speech pathologist with respect to 1) refinement of primary reconstruction for voice enhancement, 2) planning of postoperative surgical phonatory rehabilitation, and 3) planning of postoperative voice therapy.

This study reports the results of postoperative videolaryngostroboscopy in 42 patients who have undergone VPL procedures and in an additional 8 patients who have undergone endoscopic cordectomy (total n = 50), in an attempt to correlate surgical techniques with laryngeal phonatory function.

MATERIALS AND METHODS

A retrospective review of the videolaryngostroboscopic tape library of the Grabscheid Voice Center at The Mount Sinai Medical Center revealed a total of 42 patients with a history of VPL and 8 patients who had undergone endoscopic cordectomy. The patients entered into this study were not randomized and may not be representative of all VPL or endoscopic cordectomy patients in general. Rather, the subjects represent all members of a patient cohort that was alive and compliant with routine local follow-up appointments during the period of time under study. For all patients, the primary surgery and/or any subsequent reconstructive surgeries were performed by the senior authors (P.W., M.L.U., H.F.B.). Detailed operative reports and patient histories were reviewed for all patients. All videolaryngostroboscopic recordings were performed by one of the senior authors (P.W.) over the 22-month period September 20, 1996, to July 21, 1998. The recordings were obtained an average of 31.1 months (range 1 to 221 months) after the most recent ablative laryngeal surgical procedure. Videolaryngostroboscopy had been performed on all of these patients as part of routine postoperative care.

Vertical Partial Laryngectomy. Of the 42 patients who underwent VPL, 34 were men and 8 were women. The average patient age at the time of VPL was 61.8 years. Primary diagnoses are listed in Table 1. Seventeen patients had received full-course external-beam radiotherapy to the head and neck region. There

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TABLE 1. PREOPERATIVE DIAGNOSES OF 42 VPL PATIENTS

Diagnosis		No. of Patients
Glottic squamous cell carcinoma	T1a	9
	T1b	6
	T2	17
	T3	3
	Tx	2
Subglottic squamous cell carcinoma		2
Pyriform sinus squamous cell carcinoma		1
Glottic rhabdomyosarcoma		1
Glottic Teflon granuloma		1

VPL — vertical partial laryngectomy.

were 13 patients who represented primary irradiation failures, 3 patients who had received postoperative radiotherapy, and 1 patient who had received radiotherapy to the neck for a primary oral cavity carcinoma prior to the diagnosis of laryngeal carcinoma. All postoperative radiotherapy was completed 21 to 99 months prior to videolaryngostroboscopic recording.

As with most VPL series, the surgical extent of resection and the technique of reconstruction were variable.[7,8] In the present study, the VPL procedure most commonly involved excision of the majority of the ipsilateral thyroid cartilage, leaving an ipsilateral posterior cartilaginous strut attached to the inferior constrictor muscle. In 8 patients, the anterior commissure was divided in the midline, whereas in the remainder of patients, the anterior commissure and a variable amount of the anterior portion of the contralateral true vocal fold and contralateral thyroid cartilage was resected. In 4 patients, a posterior strut and a superior strut of ipsilateral thyroid cartilage were left behind. In 3 patients, thyrotomy incisions were made but thyroid cartilage was not resected, although at a later date, a vertical strip of ipsilateral thyroid cartilage was resected and used to create a neocord in 1 of these patients. The 2 patients with subglottic lesions and the 1 patient with a pyriform sinus carcinoma underwent ipsilateral hemicricoidectomy as part of the operative procedure. In 2 other patients, a 1-mm to 3-mm rim of superior cricoid cartilage was resected in order to obtain clear margins for subglottic extension. The anterior commissure technique was performed in 4 patients, in which symmetric amounts of thyroid cartilage and laryngeal soft tissue were resected bilaterally.

Primary reconstruction at the time of VPL was most commonly accomplished with pedicled strap muscle flaps sutured to the thyroid perichondrium, pyriform sinus mucosal advancement flaps, and rotation of the posterior thyroid cartilage strut onto the cricoid cartilage for lamina reconstruction (Table 2). A combination of procedures was often employed in the same patient in order to provide adequate reconstruction.

TABLE 2. PRIMARY RECONSTRUCTIVE TECHNIQUES AFTER VPL

Technique	No. of Patients
Pedicled strap muscle flap with thyroid perichondrium	31
Pyriform sinus mucosal advancement flap	11
Pedicled posterior thyroid cartilage strut rotational flap	9
Ipsilateral omohyoid flap	3
False vocal fold mucosal flap	3
Free cartilage graft	2
Skin flaps with laryngostome creation	2
Dermal graft	2
Split-thickness skin graft	1
Pedicled hyoid bone flap	1
Radial forearm free flap	1
Arytenoidopexy	1
Thyroid cartilage miniplates	12
Laryngeal stent or keel	11

Twenty patients underwent subsequent laryngeal procedures prior to videolaryngostroboscopy. The majority of these procedures were performed to relieve symptoms of airway obstruction. These procedures included endoscopic debridement of reconstructed tissue with the carbon dioxide (CO2) laser (11 patients, 16 procedures), endoscopic nonlaser debridement of granulation tissue (4 patients, 5 procedures), 3-stage laryngotracheal reconstruction (2 patients), laryngostome closure (2 patients), neocord injection with either Teflon, fat, or collagen (5 patients, 7 procedures), medialization laryngoplasty (1 patient), and contralateral endoscopic CO2 laser cordectomy (1 patient). In addition, 1 patient underwent subsequent reconstruction of a persistent anterior glottic defect with a pedicled sternohyoid myocutaneous flap inserted through a midline thyrotomy.

Endoscopic Cordectomy. Eight patients (6 men, 2 women) underwent suspension microlaryngoscopy with either CO2 laser (5 patients) or conventional (3 patients) cordectomy for glottic squamous cell carcinoma. The American Joint Committee staging classifications were the following: T1a (5 patients), T1b (1 patient), T2 (1 patient), and Tis (1 patient). No patient received radiotherapy. All procedures were performed by one of the senior authors (P.W.). One patient underwent a free buccal mucosal graft to reconstruct the true vocal fold after resection.

Three of the patients treated by endoscopic resection underwent subsequent laryngeal procedures prior

TABLE 3. VIDEOLARYNGOSTROBOSCOPIC MEASUREMENTS IN VPL PATIENTS (N = 42)

Measurements	*Patients* *No.*	*%*
Glottic closure		
Complete	7	16.7
Anterior gap	4	9.5
Posterior gap	11	26.2
Irregular gap	10	23.8
Cannot rate	10	23.8
Site of vibration		
True vocal fold	8	19.0
False vocal fold	17	40.5
Arytenoid mucosa	10	23.8
No site	7	16.7
Vibratory behavior		
Periodic	15	35.7
Rough	19	45.2
No vibration	8	19.0
Functional quality		
Patient satisfied	29	69.0
Patient wants more	13	31.0

to videolaryngostroboscopy. These included repeat endoscopic CO2 laser biopsies of the glottis (2 patients, 6 procedures) and 1 medialization laryngoplasty.

Videolaryngostroboscopy. Videolaryngostroboscopy was performed with an Olympus ENF Type P3 FOS flexible fiberoptic laryngoscope connected to a Kay RLS 9100 Rhino-Laryngeal Stroboscope light source. Images were viewed on a Mitsubishi Diamond Scan monitor and recorded onto Maxell $^1/_2$-in VHS tapes with a Mitsubishi Diamond Pro VHS tape recorder. A specific videostrobolaryngoscopy protocol was not followed, as these examinations were performed as part of routine postoperative care prior to initiation of the study. However, on review of the videotapes, it was noted that most patients had been asked to state their names, perform a variety of verbal tasks such as counting from 1 to 10, and maintain sustained production of the vowel "e." Postoperative video recordings were viewed and rated simultaneously in a nonblinded manner by 4 of the authors of the study (D.L.M., P.W., J.M., A.M.). All of these authors are experienced in endoscopy and the rating of videolaryngostroboscopic recordings. Qualitative assessments and subjective ratings were discussed for each patient, and a consensus decision was made. Glottic closure during phonation was rated as 1) complete, 2) anterior gap, 3) posterior gap, or 4) irregular gap. The predominant site of vibration was rated as 1) true vocal fold, 2) false vocal fold, 3) arytenoid mucosa, or 4) no site of vibration. Vibratory behavior was rated as 1) periodic, 2) rough, or 3) no vibration. From the video samples, vocal quality was rated on a 5-point scale as follows: 1 = normal voice, 2 = mild dysphonia, 3 = moderate dysphonia, 4 = severe dysphonia, or 5 = aphonia. Last, in an effort to measure functional vocal quality, patients were rated as being either 1) satisfied or 2) not satisfied with their voice at the time of videotaping. Patient satisfaction was determined by personal knowledge of each patient by nonblinded staff in the Voice Center, as well as by review of office records, in which patient satisfaction with voice is often noted as part of clinical record-keeping. The ratings for glottic closure, site of vibration, vibratory behavior, and functional quality, as demonstrated above, were recorded in the form of categorical data. The χ^2 test was chosen to search for statistically significant differences between the distribution of categorical results (for example, to search for a difference between the distribution of sites of vibration between true vocal fold, false vocal fold, and arytenoid mucosa) between any 2 given groups of patients. Subjective voice quality ratings, on the other hand, represented continuous numerical data, for which a mean score could be calculated for any given group of patients. Statistically significant differences between vocal quality mean scores were determined with Student's *t*-test when 2 groups were being compared, and with a 1-way ANOVA when 3 groups were being compared. A 2-sided α level of 0.05 was considered statistically significant for all tests. The statistics program utilized was SPSS 8.0 for Windows.

RESULTS

The results for the 42 patients who underwent VPL are presented in Table 3. Complete glottic closure was achieved in a minority of patients (16.7%). Mucosal vibration during phonation was completely absent in 7 (16.7%) patients (most commonly due to an inability of contralateral laryngeal mucosa to appose reconstructed laryngeal tissue). Among the remaining 35 patients, the site of mucosal vibration upon phonation was most commonly noted at the contralateral false vocal fold (17 patients), followed by the contralateral arytenoid mucosa (10 patients) and, least commonly, at the contralateral true vocal fold (8 patients). A one-way ANOVA was used to compare the mean vocal quality scores among 3 groups of patients: those with true vocal fold vibration, those with false vocal fold vibration, and those with arytenoid mucosal vibration. Among these 3 sites of vibration, there was no statistically significant difference in the mean vocal quality (Table 4).

VPL With Arytenoidectomy Versus VPL Without Arytenoidectomy. Among the 42 patients who underwent VPL procedures, 21 patients (50.0%) underwent ipsilateral arytenoidectomy and 21 patients

TABLE 4. MEAN VOCAL QUALITY SCORE FOR EACH SITE OF VIBRATION AMONG VPL PATIENTS (PATIENTS WITH NO SITE OF VIBRATION EXCLUDED)

Site of Vibration	*Mean*	*SD*	*No. of Patients*
True vocal fold	3.63	0.52	8
False vocal fold	3.29	0.59	17
Arytenoid mucosa	3.60	0.84	10
Total	3.46	0.66	35

p = .373 (ANOVA).

(50.0%) had their arytenoid cartilages preserved. The videolaryngostroboscopic data for each of these 2 groups are presented in Tables 5 and 6. Significant differences were noted between the 2 groups with respect to the overall distribution of the sites of vibration during phonation (χ^2 test, p = .019). Within the arytenoidectomy group, the most common site of vibration was the contralateral arytenoid mucosa, while only 1 patient demonstrated true vocal fold vibration. In contrast, in the nonarytenoidectomy group, the most common site of vibration was the contralateral false vocal fold, followed closely by the contralateral true vocal fold and, last, the contralateral arytenoid mucosa (only 2 patients). There was no statistically significant difference between the 2 groups with respect to glottic closure, vibratory behavior, mean vocal quality, or patient satisfaction with voice.

TABLE 5. VIDEOLARYNGOSTROBOSCOPIC EFFECT OF ARYTENOIDECTOMY IN VPL PATIENTS (N = 42)

	With Arytenoidectomy (n = 21)		*Without Arytenoidectomy (n = 21)*		
Measurements	*No.*	*%*	*No.*	*%*	*p (χ^2)*
Glottic closure					.496
Complete	2	9.5	5	23.8	
Anterior gap	2	9.5	2	9.5	
Posterior gap	7	33.3	4	19.0	
Irregular gap	6	28.6	4	19.0	
Cannot rate*	4	19.0	6	28.6	
Site of vibration					.019
True vocal fold	1	4.8	7	33.3	
False vocal fold	7	33.3	10	47.6	
Arytenoid mucosa	8	38.1	2	9.5	
No site	5	23.8	3	14.3	
Vibratory behavior					.079
Periodic	4	19.0	11	52.4	
Rough	12	57.1	7	33.3	
None	5	23.8	2	9.5	
Functional quality					.095
Patient satisfied	17	81.0	12	57.1	
Wants more	4	19.0	9	42.9	

*Excluded from χ^2 analysis.

TABLE 6. EFFECT OF ARYTENOIDECTOMY ON VOCAL QUALITY SCORE IN VPL PATIENTS (N = 42)

Arytenoidectomy	*N*	*Mean*	*SD*
Yes	21	3.71	0.84
No	21	3.62	0.80
Total	42	3.67	0.82

p = .710 (Student's *t*-test).

Radiotherapy. Among the 42 patients who underwent VPL, 17 patients (40.5%) had undergone full-course external-beam radiotherapy to the larynx or neck, while 25 patients (59.5%) had never received radiotherapy to the head and neck region. Videostrobolaryngoscopic data for each of these 2 groups are presented in Tables 7 and 8.

There was no statistically significant difference between groups with respect to glottic closure measurements, site of vibration, or functional quality, on the χ^2 test for categorical data. However, the number of patients who demonstrated periodic mucosal vibratory behavior was a significantly greater number in the nonradiotherapy group than in the radiotherapy group (χ^2 test, p = .025; Table 7).

The mean vocal quality score for the radiotherapy group was 3.94, indicating more severe dysphonia than for the nonradiotherapy group, which had a mean of 3.48 (Table 8). The difference between these means approached, but did not achieve, statistical significance (Student's *t*-test, p = .072).

Pyriform Sinus Mucosal Flap. Among the 21 pa-

TABLE 7. VIDEOLARYNGOSTROBOSCOPIC EFFECT OF RT ON VPL PATIENTS (N = 42)

	With RT (n = 17)		*Without RT (n = 25)*		
Measurements	*No.*	*%*	*No.*	*%*	*p (χ^2)*
Glottic closure					.395
Complete	2	11.8	5	20.0	
Anterior gap	2	11.8	2	8.0	
Posterior gap	5	29.4	6	24.0	
Irregular gap	7	41.2	3	12.0	
Cannot rate*	1	5.9	9	36.0	
Site of vibration					.128
True vocal fold	3	17.6	5	20.0	
False vocal fold	4	23.5	13	52.0	
Arytenoid mucosa	7	41.2	3	12.0	
No site	3	17.6	4	16.0	
Vibratory behavior					.025
Periodic	2	11.8	13	52.0	
Rough	10	58.8	9	36.0	
None	5	29.4	3	12.0	
Functional quality					.237
Patient satisfied	10	58.8	19	76.0	
Wants more	7	41.2	6	24.0	

RT — radiotherapy.
*Excluded from χ^2 analysis.

TABLE 8. EFFECT OF RT ON VOCAL QUALITY SCORE IN VPL PATIENTS (N = 42)

RT	*N*	*Mean*	*SD*	*SE*
Yes	17	3.94	0.83	0.20
No	25	3.48	0.77	0.15
Total	42	3.67	0.82	0.13

p = .072 (Student's *t*-test).

tients who underwent VPL with arytenoidectomy, a pyriform sinus mucosal flap was utilized for laryngeal reconstruction in 10 patients (47.6%), and not utilized in 11 patients (52.4%). The videolaryngostroboscopic data for each of these 2 groups are presented in Tables 9 and 10. No statistical differences were noted between groups with respect to glottic closure measurements, site of vibration, vibratory behavior, mean vocal quality scores, or patient satisfaction with voice.

Endoscopic Cordectomy. The videostrobolaryngoscopic data for the 8 patients who underwent endoscopic cordectomy and the 42 patients who underwent VPL are presented in Tables 11 and 12. Endoscopic cordectomy patients performed better than VPL patients with respect to all measured parameters.

DISCUSSION

Vertical partial laryngectomy techniques have been well described and popularized as a means of eradicating select laryngeal carcinomas while attempting to preserve the basic laryngeal functions of respiration, prevention of aspiration, and phonation.[1-5] Reconstruction of the post-VPL defect involves replacement of tissue bulk to provide a surface against which contralateral mucosa can vibrate and produce voice. Most reconstructive techniques involve the use of local muscle, mucosal, perichondrial, or cartilage flaps.[6]

TABLE 9. VIDEOLARYNGOSTROBOSCOPIC EFFECT OF PYRIFORM MUCOSAL FLAP RECONSTRUCTION IN PATIENTS WHO UNDERWENT VPL WITH ARYTENOID RESECTION

	With Pyriform Flap (n = 10)		*Without Pyriform Flap (n = 11)*		
Measurements	*No.*	*%*	*No.*	*%*	*p (χ^2)*
Glottic closure					.962
Complete	1	10.0	1	9.1	
Anterior gap	1	10.0	1	9.1	
Posterior gap	3	30.0	4	36.4	
Irregular gap	2	20.0	4	36.4	
Cannot rate*	3	30.0	1	9.1	
Site of vibration					.400
True vocal fold	0	0.0	1	9.1	
False vocal fold	5	50.0	2	18.2	
Arytenoid mucosa	3	30.0	5	45.5	
No site	2	20.0	3	27.3	
Vibratory behavior					.926
Periodic	2	20.0	2	18.2	
Rough	6	60.0	6	54.5	
None	2	20.0	3	27.3	
Functional quality					.314
Patient satisfied	9	90.0	8	72.7	
Wants more	1	10.0	3	27.3	

*Excluded from χ^2 analysis.

TABLE 10. EFFECT OF PYRIFORM SINUS MUCOSAL FLAP ON VOCAL QUALITY SCORE IN VPL PATIENTS WITH ARYTENOIDECTOMY (N = 21)

Pyriform Flap	*N*	*Mean*	*SD*
Yes	10	3.60	0.27
No	11	3.82	0.26

p = .568 (Student's *t*-test).

The utility of videostroboscopy for clinical evaluation of the larynx has been well described.[9] The technique utilizes pulses of light that intermittently illuminate the vocal folds. Fusion of these images by the human eye results in perceived mucosal motion. In the normal larynx, videostroboscopy can be used to observe such characteristics as vocal fold symmetry, periodicity and amplitude of vocal fold vibration, glottic closure, vocal fold mucosal wave, and vocal fold stiffness.

In this study, videolaryngostroboscopy was performed in 42 patients who had previously undergone VPL procedures. Phonation in the majority of VPL patients appeared to originate from vibratory activity at sites other than the glottis. Hirano et al[7] in 1987 demonstrated that patients who had undergone ex-

TABLE 11. VIDEOLARYNGOSTROBOSCOPIC EFFECT OF ENDOSCOPIC CORDECTOMY COMPARED TO VPL

	VPL (n = 42)		*Cordectomy (n = 8)*		
Measurements	*No.*	*%*	*No.*	*%*	*p (χ^2)*
Glottic closure					.009
Complete	7	16.7	6	75.0	
Anterior gap	4	9.5	2	25.0	
Posterior gap	11	26.2			
Irregular gap	10	23.8			
Cannot rate*	10	23.8			
Site of vibration					.000
True vocal fold	8	19.0	8	100.0	
False vocal fold	17	40.5			
Arytenoid mucosa	10	23.8			
No site	7	16.7			
Vibratory behavior					.004
Periodic	15	35.7	8	100.0	
Rough	19	45.2			
None	8	19.0			
Functional quality					.093
Patient satisfied	29	69.0	8	100.0	
Wants more	13	31.0			

*Excluded from χ^2 analysis.

TABLE 12. COMPARISON OF MEAN VOCAL QUALITY SCORES BETWEEN ENDOSCOPIC CORDECTOMY PATIENTS AND VPL PATIENTS

Cordectomy	*N*	*Mean*	*SD*
Yes	8	2.12	0.35
No	42	3.67	0.82
Total	50	3.42	0.95

$p < .0001$ (Student's *t*-test).

tended frontolateral laryngectomy with subsequent reconstruction commonly demonstrated vibration of nonglottic sites, particularly at the false vocal fold (55% of patients). It was also shown that maximum phonation time was decreased, while mean airflow rate during phonation was increased, compared to normative data. In that report, complete glottic closure was achieved in only 19% of patients.[7] In the present study, only 16.7% of VPL patients demonstrated complete glottic closure.

In the present study, vocal quality scores were statistically similar among VPL patients, regardless of the location of the vibratory source. The most common rating after VPL was "moderate dysphonia."

Among VPL patients, arytenoidectomy significantly changed the site of vibration during phonation. Arytenoidectomy patients demonstrated more contralateral arytenoid mucosal vibration and less true vocal fold vibration when compared to VPL patients in whom the arytenoid cartilages had been preserved. Using videolaryngoscopy, Blaugrund et al[8] reported supraglottic involvement in voice production to be a predominant finding in VPL patients in whom 1 arytenoid had been resected as compared to those patients in whom the arytenoid cartilages had been spared.

In the present study, among patients who underwent VPL with arytenoidectomy, the use of the pyriform sinus mucosal advancement flap did not affect mean vocal quality (p = .568, Table 10). Reconstructive techniques other than the pyriform sinus mucosal flap were utilized either too frequently or too infrequently (Table 2) to be subjected to a meaningful statistical evaluation with respect to their effects on vocal quality.

The majority of VPL patients in the present study (29/42 or 69.0%) were satisfied with their voices at the time of videolaryngostroboscopy. Despite ratings of "moderate" or "severe" dysphonia, most patients were able to use their voice satisfactorily in daily activities and did not seek further voice improvement. These findings are echoed in a study by Schuller et al,[10] in which 12 of 12 patients who had undergone vertical hemilaryngectomy with pyriform sinus mucosal reconstruction reported excellent or satisfactory voice on a self-assessment questionnaire.

Among those VPL patients who received radiotherapy in the present study, a significant decrease in periodic mucosal vibration upon phonation was noted when compared to nonirradiated patients (χ^2 test, p = .025), as was a trend toward poorer vocal quality (Student's *t*-test, p = .072). These findings are similar to those noted by Lehman et al,[11] in which 20 patients treated solely with radiotherapy for early glottic carcinoma commonly displayed vocal fold stiffness on videolaryngostroboscopy, in addition to poor voice production.

Endoscopic cordectomy, either with the CO2 laser as first described by Strong and Jako[12] or with standard microsurgical techniques, has been shown to be a viable treatment option in patients with early-stage glottic carcinoma.[13,14] In a study by Casiano et al,[15] videolaryngostroboscopy of 6 post–CO2 laser cordectomy patients demonstrated an impaired mucosal wave and incomplete glottic closure, with moderate to breathy dysphonia. However, in a study by Schuller et al,[10] 5 of 5 patients who underwent endoscopic CO2 laser cordectomy were satisfied with their postoperative voices. It was noted by Keilmann et al[16] that laser cordectomy patients more often phonated on a purely glottic level, with better vocal quality, as compared to patients who had conventional cordectomy, although nearly all patients in both groups were satisfied with their postoperative vocal quality.

In the present study, although the treated vocal fold appeared stiff after CO2 laser cordectomy, complete glottic closure was achieved, with periodic vibratory activity noted in the contralateral fold. An anterior glottic gap was noted upon phonation in 2 of 3 nonlaser endoscopic cordectomy patients. Vocal quality ratings made after endoscopic cordectomy ranged from mild to moderate dysphonia, and all patients were satisfied with their postoperative voices. The 8 endoscopic cordectomy patients in this study performed significantly better than the 42 VPL patients with respect to all measured parameters.

CONCLUSION

This study highlights some important features of VPL as it relates to voice reconstruction. First, it should be noted that VPL is an oncologically satisfactory surgical procedure, even after irradiation failure. Second, despite a suboptimal postoperative voice, the majority of patients were nonetheless satisfied with their phonatory ability. The opportunity to live without a stoma, with a normal swallow and some residual dysphonia, was accepted by patients overwhelmingly over other alternatives. Only in highly demanding vocal professions (ie, executives, actors, dentists, doctors) were further voice restoration procedures sought after hemilaryngectomy. Third, vocal reconstruction after VPL and extended VPL remains a surgical challenge. That few post-VPL patients had normal glottic vibration points to the difficulty of restoring pliable anatomic structures resected at surgery. During reconstruction, soft, pliable tissue may be provided by a pyriform sinus mucosal flap, false vocal fold imbrication, or a buccal mucosal graft, all of which represent examples of tissue that may be more pliable than a standard muscle-perichondrial flap. Furthermore, care must be exercised after surgery to avoid resecting new vibratory structures in an attempt to better visualize the glottis.

Finally, rehabilitation by voice therapy should aim to produce a glottal sound source if possible. However, phoniatric therapy to promote false vocal fold vibration and arytenoid-to-epiglottis sources of vibration can produce very satisfactory phonatory results.

This study demonstrates that laryngeal reconstruction after VPL provides the soft tissue bulk necessary for mucosal contact and vibration upon phonation. Videolaryngostroboscopic recordings demonstrated that nonglottic phonatory sources (ie, false vocal fold, arytenoid mucosa) are often responsible for the successful phonation observed after VPL.

REFERENCES

1. Som ML. Hemilaryngectomy: modified technique for cordal carcinoma with extension posteriorly. Arch Otolaryngol 1951;54:524-30.

2. Som ML, Silver CE. The anterior commissure technique of partial laryngectomy. Arch Otolaryngol 1968;87:138-45.

3. Ogura JH, Biller HF. Conservative surgery in cancer of the head and neck. Otolaryngol Clin North Am 1969;2:641-65.

4. Biller HF, Ogura JH, Pratt LL. Hemilaryngectomy for T2 glottic cancers. Arch Otolaryngol 1971;93:238-43.

5. Biller HF, Lawson W. Partial laryngectomy for vocal cord cancer with marked limitation or fixation of the vocal cord. Laryngoscope 1986;96:61-4.

6. Bailey BJ, Stiernberg CM. Newer treatments for glottic cancer. In: Myers EN, Bluestone CD, Brackmann DE, Krause CT, eds. Advances in otolaryngology–head and neck surgery. Vol 2. Chicago, Ill: Year Book Medical Publishers, Inc, 1988: 105-40.

7. Hirano M, Kurita S, Matsuoka H. Vocal function following hemilaryngectomy. Ann Otol Rhinol Laryngol 1987;96:586-9.

8. Blaugrund SM, Gould WJ, Haji T, Meltzer J, Bloch C, Baer T. Voice analysis of the partially ablated larynx. A preliminary report. Ann Otol Rhinol Laryngol 1984;93:311-7.

9. Bless D, Hirano M, Feder RJ. Videostroboscopic evaluation of the larynx. Ear Nose Throat J 1987;66:289-96.

10. Schuller DE, Trudeau M, Bistline J, LaFace K. Evaluation of voice by patients and close relatives following different laryngeal cancer treatments. J Surg Oncol 1990;44:10-4.

11. Lehman JJ, Bless DM, Brandenburg JH. An objective assessment of voice production after radiation therapy for stage I squamous cell carcinoma of the glottis. Otolaryngol Head Neck Surg 1988;98:121-9.

12. Strong MS, Jako GJ. Laser surgery in the larynx. Early clinical experience with continuous CO_2 laser. Ann Otol Rhinol Laryngol 1972;81:791-8.

13. Blakeslee D, Vaughan CW, Shapshay SM, Simpson GT, Strong MS. Excisional biopsy in the selective management of T1 glottic cancer: a three-year follow-up study. Laryngoscope 1984;94:488-94.

14. Cragle SP, Brandenburg JH. Laser cordectomy or radiotherapy: cure rates, communication, and cost. Otolaryngol Head Neck Surg 1993;108:648-54.

15. Casiano RR, Cooper JD, Lundy DS, Chandler JR. Laser cordectomy for T1 glottic carcinoma: a 10-year experience and videostroboscopic findings. Otolaryngol Head Neck Surg 1991; 104:831-7.

16. Keilmann A, Bergler W, Artzt M, Hormann K. Vocal function following laser and conventional surgery of small malignant vocal fold tumors. J Laryngol Otol 1996;110:1138-41.

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ELECTRON MICROSCOPIC AND IMMUNOHISTOCHEMICAL INVESTIGATION OF REINKE'S EDEMA

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Examination was made of the vocal fold mucosa of Reinke's edema immunohistochemically and electron microscopically, with special attention to blood vessels in Reinke's space (RS). Ten surgical specimens of Reinke's edema were used, and the results obtained are summarized as follows. 1) There were subepithelial vascularization and many dilated vessels in RS. 2) The vessels had thin endothelium with many fenestrae, many vesicles, and a thickened basement membrane. 3) Plasma exuded from the capillaries into surrounding tissue via fenestrae. 4) These findings demonstrate increased permeability of the vessels. 5) The vascular walls were thin, with few pericytes. 6) The pericytes were situated away from the endothelial cells. 7) Some endothelial cells and pericytes had degenerated, and vessel occlusion was apparent in some cases. 8) These findings indicate the vessels had become fragile. 9) Interstitial cells and/or inflammatory cells in RS showed stained cytoplasm with vascular endothelial growth factor. 10) This growth factor may possibly enhance capillary permeability and form fragile vessels in RS. Fragility and alteration in permeability of the vessels are presumed to cause edema of RS, which may progress to Reinke's edema.

KEY WORDS — electron microscopic study, immunohistochemical study, Reinke's edema, vascular endothelial growth factor.

INTRODUCTION

Reinke's edema is a common disease of the vocal fold ultimately causing changes in voice quality. The entire length of the membranous vocal fold is edematous and swollen.[1] Histologically, the primary feature is edema in Reinke's space.[1] The most frequent etiologic factors of Reinke's edema are considered to be smoking and aging.[2] The mechanism for the onset and development of the disease remains unclear.

During endolaryngeal microsurgery of Reinke's edema, subepithelial vascularization is seen in the vocal fold mucosa. The vessels are not parallel to the edge of the vocal fold, but run in random directions and are particularly conspicuous in severe cases. Edema in Reinke's space appears to be related to blood vessels in this space.

Examination was made of the vocal fold mucosa of Reinke's edema immunohistochemically and electron microscopically, with special attention to blood vessels in the superficial layer of the lamina propria (Reinke's space). Based on the results obtained, a mechanism for onset and subsequent development of this disease is discussed.

MATERIALS AND METHODS

Ten vocal fold specimens with Reinke's edema obtained during endolaryngeal microsurgery and 3 normal vocal fold specimens from autopsy cases were used.

For transmission electron microscopy (TEM), 3 surgical specimens of Reinke's edema and 3 normal vocal folds were fixed in 2.5% glutaraldehyde at 4°C for 2 hours, rinsed with cacodylate buffer solution, and postfixed in 2% osmium tetroxide at 4°C for 2 hours. These specimens were dehydrated in a graded series of concentrations of ethanol and embedded in epoxy resin. Semithin sections were prepared with an ultramicrotome, stained with 1% toluidine blue, and examined with a light microscope. Thin sections were made with an ultramicrotome and stained with uranyl acetate and lead citrate and tannic acid stain. Observation was conducted with a JEM-2000-EX transmission electron microscope.

The vascular endothelial growth factor (VEGF) was detected histologically in formalin-fixed and paraffin-embedded tissue by immunohistochemistry. This growth factor was used since it specifically acts on endothelial cells of vessels. For immunohistochemical study, a labeled streptavidin-biotin staining kit (DAKO, USA) was used. Seven paraffin-embedded surgical specimens of Reinke's edema and 3 normal vocal folds were sectioned at 5- to 6-µm thickness and mounted on glass slides. Deparaffinized and hydrated sections were rinsed with 0.01-mol/L phosphate buffered saline (PBS) at pH 7.4 and treated with 0.1% trypsin (Sigma) in Tris-buffered

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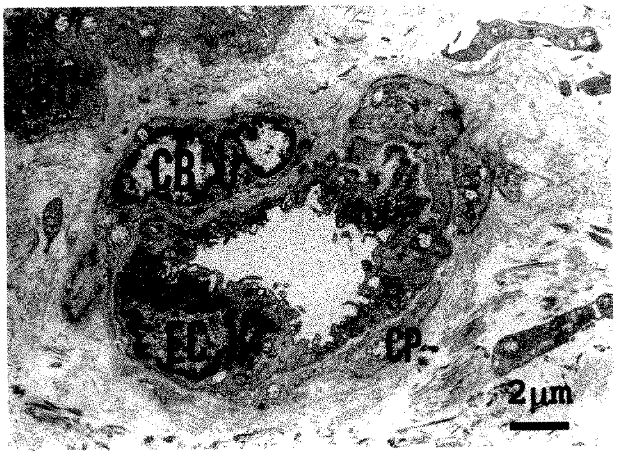

Fig 1. Capillary in Reinke's space of normal vocal fold mucosa. EC — endothelial cell, CB — cell body of pericyte, CP — cytoplasmic process of pericyte, BC — basal cell of epithelium.

saline for 30 minutes at 37°C. The specimens were covered with 3% hydrogen peroxide for 5 minutes and rinsed with 0.01-mol/L PBS, followed by treatment with normal goat serum. The specimens were then incubated with the primary antibody overnight at 4°C. A 1:500 diluted polyclonal antibody against VEGF (Oncogene Science, Inc, Cambridge, Mass) was used. After rinsing with PBS and labeling with a streptavidin-biotin staining kit, a color reaction was developed with 3,3'-diaminobenzidine for about 10 minutes at room temperature. Immunoreactivity was examined by light microscopy.

RESULTS

Figure 1 shows a capillary in the superficial layer of the lamina propria (Reinke's space) of normal vocal fold mucosa. There were only small vessels, such as capillaries, arterioles, and venules, in this space. The capillaries were about 10 µm in diameter (Fig 1). Cell bodies and processes of the pericytes encircled the capillaries and were attached to their walls (Fig 1). The cell bodies and endothelial cells remained separated by a gap of 300 to 500 nm. The processes were in close contact with endothelial cells, sharing a common basement membrane with endothelial cells.

Figure 2 shows blood vessels in the superficial layer of lamina propria (Reinke's space) of the vocal fold mucosa in Reinke's edema. Subepithelial vascularization was evident in Reinke's space. Blood vessels were dilated and 20 to 30 µm in diameter (Fig 2A,B). The blood vessel walls were thin (Fig 2A,B), as was the cytoplasm of the endothelial cells (Fig 2A,B). The cytoplasm (cell body and branching cytoplasmic processes) of the pericytes was thin (Fig 2A,B). The cell bodies and branching processes were not attached to the endothelial cells of the vessels (Fig 2A). The pericytes were situated away from the endothelial cells, and shared the thickened basement membrane with them (Fig 2A). The number of pericytes had decreased.

Many vesicles were present in the cytoplasm of the endothelial cells of the vessels, and the endothelial cells possessed many fenestrae (Fig 2C). Plasma exuded from the capillaries into surrounding tissue via the fenestrae (Fig 2D). Thickened basement membrane and dense reticular fibers were noted around the vessels (Fig 2B,C). Capillary permeability thus appeared to be increased in Reinke's edema.

The endothelial cells and pericytes of some vessels were degenerated, and partial or complete vessel occlusion was seen in some cases (Fig 2E,F).

Figure 3 shows the immunohistochemical findings of VEGF in the superficial layer of the lamina propria (Reinke's space) of the vocal fold mucosa in Reinke's edema. In 5 of the 7 cases, interstitial cells (Fig 3A,B) and/or inflammatory cells (Fig 3C) in the superficial layer of the lamina propria (Reinke's space) of the vocal fold mucosa showed cytoplasm staining with VEGF, while this growth factor stained no component in the lamina propria of the normal vocal folds.

DISCUSSION

Reinke's edema has several different names, such as polypoid vocal fold, polypoid degeneration, chronic polypoid corditis, and chronic edematous hypertrophy.[1] Histologically, the primary feature of this disease is edema in the superficial layer of the lamina propria or Reinke's space.[1] The pathology is not degeneration, inflammation, or hypertrophy.[1] The mechanism for the onset and development of Reinke's edema remains unclear.

The blood vessels are unique at the normal vocal fold edge, where only small vessels such as capillaries, arterioles, and venules are present.[3-6] The vessels enter the vocal fold edge from the anterior or posterior end of the membranous vocal fold and run essentially parallel to the edge. Their structure is suited for vibration.[3-5] Pericytes surround the capillaries in the vocal fold mucosa and provide mechanical support and protection to the capillary walls, particularly during phonation.[6]

Marked pathological changes in vessels in the superficial layer of the lamina propria (Reinke's space) were noted in this study. Many dilated vessels were present in Reinke's space, and telangiectasia was a major feature of the disease. The capillary diameter varied considerably, even in the middle portion of the vocal fold mucosa. The vessels had thin endothe-

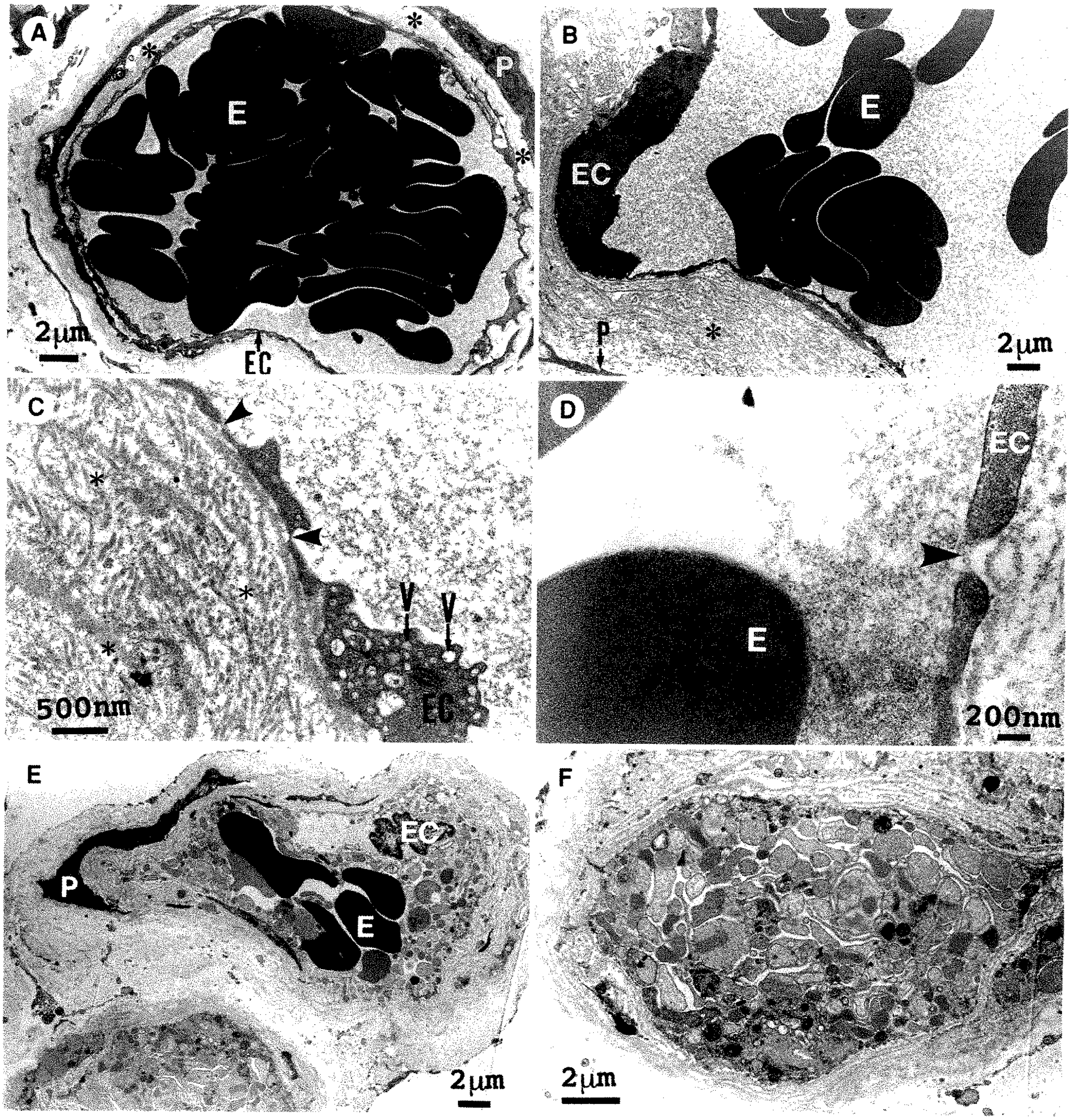

Fig 2. Transmission electron microscopy of Reinke's space of vocal fold mucosa in Reinke's edema. EC — endothelial cell, P — pericyte, E — erythrocyte, asterisks — thickened basement membrane of endothelial cells. A-D) Dilated blood vessels. V — vesicles, arrowheads — fenestrae. D) Fenestra of vessel. Arrowhead — fenestra and plasma exuding from capillary. E,F) Degenerated and occluded vessels.

lium with many fenestrae and vesicles and thickened basement membrane, indicating increased permeability. Plasma was found to exude from the capillaries into surrounding tissue via the fenestrae. Vascular wall thinning and a reduction in pericytes were also apparent. The pericytes were situated away from the endothelial cells. The pericytes appeared not to adequately support and protect the capillary walls, particularly during phonation. Some endothelial cells and pericytes had degenerated, and vessel occlusion was sometimes seen. Thus, the vessels in Reinke's edema are fragile, and this fragility may affect the blood circulation in the vocal fold mucosa.

The VEGF, also known as vascular permeability factor, is a secreted heparin-binding cytokine. The VEGF has been implicated in the control of angiogenesis due to its selective mitogenic stimulation of vascular endothelial cells and enhancement of vascular permeability.

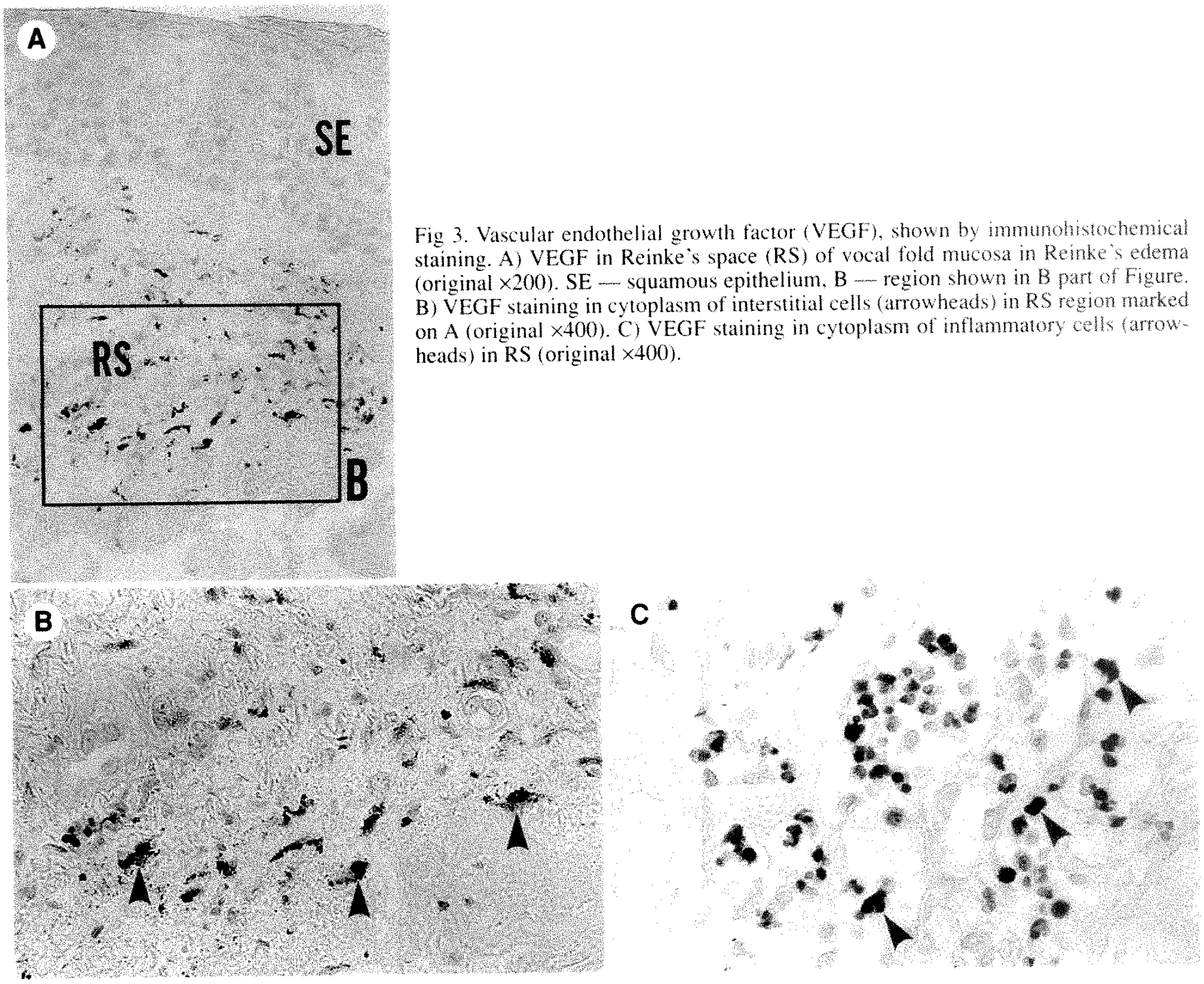

Fig 3. Vascular endothelial growth factor (VEGF), shown by immunohistochemical staining. A) VEGF in Reinke's space (RS) of vocal fold mucosa in Reinke's edema (original ×200). SE — squamous epithelium, B — region shown in B part of Figure. B) VEGF staining in cytoplasm of interstitial cells (arrowheads) in RS region marked on A (original ×400). C) VEGF staining in cytoplasm of inflammatory cells (arrowheads) in RS (original ×400).

Subepithelial vascularization, telangiectasia of the vessels, and increased permeability of the vessels were observed in this study. In 5 of 7 cases, the interstitial cells and/or inflammatory cells in the lamina propria showed cytoplasm stained with VEGF. The absence of staining in the other cases may indicate protein degeneration during collection and processing, or sampling outside the time period of maximal VEGF production. The lamina propria of the normal vocal folds showed no staining by VEGF.

The VEGF produced by interstitial cells and/or inflammatory cells in Reinke's space may promote vascularization and an increase in vessel permeability.

The most frequent etiologic factors of Reinke's edema are considered to be smoking and aging.[2] Another possible etiologic factor is vocal abuse. Smoking causes the blood flow through the vessels to decrease. It increases platelet aggregation[7] and changes blood lipid and lipoprotein concentrations.[8] It also increases carboxyhemoglobin,[9] with a consequent reduction in the ability of hemoglobin to deliver oxygen to tissue. Smoking enhances the possibility of thrombosis in the vocal fold mucosa. These deleterious effects of smoking lead to hypoxia and ischemia and affect the blood circulation in the vocal fold mucosa.

Age-related changes in the vessels increase the possibility of thrombosis and decrease blood flow through the vessels, thus also leading to hypoxia and ischemia.

Even in normal vocal folds, the blood flow of the vocal fold mucosa is reduced during phonation.[10] Fragile vessels not parallel to the edge of the vocal fold but running in random directions in Reinke's space are easily injured and collapse during phonation. The vibration patterns of the edematous vocal folds of Reinke's edema have changed, with an additional adverse effect on fragile vessels.

These etiologic factors bring about hypoxia and ischemia of the vocal fold mucosa. Hypoxia in vitro and ischemia in vivo increase VEGF messenger RNA in normal tissues and in certain human tumors.[11] These disorders may increase VEGF, with possibly greater subepithelial vascularization and capillary permeability in Reinke's space.

Thus, fragility of and alteration in the permeability of the vessels are presumed to cause edema of the superficial layer of the lamina propria (Reinke's space), which may progress to Reinke's edema.

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REFERENCES

1. Hirano M. Surgical anatomy and physiology of the vocal folds. In: Gould WJ, Sataloff RT, Spiegel JR, eds. Voice surgery. St Louis, Mo: Mosby, 1993:145-6.
2. Sanada T, Tanaka S, Sato K, Hirano M. Reinke's edema. A ten year review of 198 patients. Pract Otol (Kyoto) 1993;(suppl 62):27-32.
3. Hirano M. Phonosurgery. Basic and clinical investigations. Otologia (Fukuoka) 1975;21(suppl 1):254-60.
4. Hirano M, Sato K. Histological color atlas of the human larynx. San Diego, Calif: Singular Publishing Group, 1993:106-7.
5. Mihashi S, Okada M, Kurita S, Nagata K, Hirano M, Nakashima T. Vascular network of the vocal fold. In: Stevens KN, Hirano M, eds. Vocal fold physiology. Tokyo, Japan: University of Tokyo Press, 1981:45-59.
6. Sato K, Hirano M. Fine three-dimensional structure of pericytes in the vocal fold mucosa. Ann Otol Rhinol Laryngol 1997;106:490-4.
7. Hawkins RL. Smoking, platelets and thrombosis. Nature 1972;236:450-2.
8. Craig WY, Palomaki GE, Haddow JE. Cigarette smoking and serum lipid and lipoprotein concentration: an analysis of published data. BMJ 1989;298:784-8.
9. Brody JS, Coburn RF. Carbon monoxide–induced arterial hypoxemia. Science 1969;164:1297-8.
10. Hiroto I, Toyozumi Y, Tomita H, et al. An experimental study on the hemodynamics of the vocal fold during vibration. J Otolaryngol Jpn 1969;72:884-8.
11. Sheveiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 1992;359:843-5.

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CHARACTERIZATION OF HUMAN PAPILLOMAVIRUS IN AIRWAY PAPILLOMAS BY HISTOLOGIC AND BIOCHEMICAL ANALYSIS

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The role of human papillomavirus (HPV) in airway papillomas has been well defined in recent literature. The chronicity and recurrence of papillomas has been postulated to be a result of residual viral genome in tissue treated with standard laser techniques. Thirteen patients with airway papillomas were selected for study with polymerase chain reaction (PCR) methods to detect viral DNA. Specimens taken prior to laser therapy and specimens taken at laser margins were consistently positive for HPV DNA by PCR. The HPV DNA is apparently present in tissues after macroscopic disease has been ablated by laser techniques. Histologic analysis of laser biopsies demonstrated fragments of squamous epithelium with cytologic features of HPV infection. Laser treatment is ineffective in eradicating HPV-infected tissues from airway papillomas, and this finding supports the notion that recurrence is a product of HPV incorporated into tissue not ablated by laser irradiation. Specific methods, results, and clinical correlation will be discussed.

KEY WORDS — human papillomavirus, recurrent respiratory papillomatosis.

INTRODUCTION

Recurrent respiratory papillomatosis (RRP) remains a challenging and often frustrating disease entity to treat. Despite significant advances in the molecular-biologic characterization of the disease process and potential medical therapies, the mainstay of treatment has remained surgical extirpation.[1] At present, laser therapy of recurrent papillomas via the aerodigestive tract represents the state-of-the-art surgical therapy. Enhancement of laser therapy with additional medical methods such as interferon has been proven modest.[2]

During the clinical course of RRP, certain sites of the respiratory tract have a predilection for infection and recurrence. These areas of the respiratory tract contain squamous epithelium either as a normal anatomic location (ie, true vocal cords) or as a result of trauma, irritation, or surgical exposure.[3] Studies have demonstrated that the human papillomavirus (HPV) establishes itself in the basal layer of squamous epithelium, probably after one of the aforementioned incidents. Following infection, there is a stimulation of cell growth and a change in the normal cell maturation. These alterations manifest in the formation of a papilloma. Molecular and biochemical techniques have demonstrated the presence of viral DNA and viral protein at various levels of the squamous epithelium within papillomas.[4,5] Viral DNA, most likely representing a latent form of the virus, has also been detected in apparently normal tissues throughout the aerodigestive tract in patients with RRP. The complicating factor in RRP has been postulated to be a product of this latent form of the virus that can remain hidden within the clinically normal mucosa.[6]

The mechanism by which the HPV recurs following surgical therapy is still not well understood. Recurrence may result from multiple factors; there may be incomplete removal, stimulation of latent virus, or new infection. Our studies are an examination of the molecular-biological and histologic features of papilloma resection sites at the time of surgery.

MATERIALS AND METHODS

Patient Selection. Thirteen patients were selected from patient groups of 4 separate surgeons from the University of Kansas Medical Center. Each patient had previously received a diagnosis of recurrent papillomatosis of the upper aerodigestive tract requiring repeated surgical therapy. Following consent for sample collection, each patient was treated with laser therapy in the standard fashion as directed by the surgeon. Biopsy specimens were removed before microlaryngeal laser surgery and after surgery at the laser bed margin. All samples were immediately frozen in liquid nitrogen and DNA was extracted at a later time; some samples were formalin-fixed and paraffin-treated for histologic analysis. Laser therapy was performed under direct laryngoscopy with either

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TABLE 1. PAPILLOMA PATIENT PROFILE

Patient No.	*Age (y)*	*Age of Onset*	*No. of Surgeries*	*Papilloma Status*	*Site*	*Adjuvant Therapy*	*Sex*
1	2	1	12	Active	SUP, G	IF	M
2	3	2	9	Active	G, SUB	None	F
3	3	1.5	6	Active	G	None	M
4	3	3	4	Active	G	None	F
5	4	1.5	20	Active	G, SUB	None	F
6	4.5	2	50	Active	SUP, G, SUB, tracheal	IF, VIT	M
7	6	4	15	Active	G, SUB	None	F
8	20	15	12	Active	SUP, G	None	F
9	25	4	128	Active	SUP, G, SUB	None	M
10	34	32	4	Active	Nasal	None	M
11	36	32	5	Active	G, SUB	AC, VIT	M
12	38	3	70	Active	G	None	F
13	39	34	4	Active	SUP, G	AC	F

SUP — supraglottic, G — glottic, IF — interferon, SUB — subglottic, VIT — "cruciferous" vitamins, AC — acyclovir.

a Sharplan carbon dioxide laser at a wavelength of 10.6 µm or a Laser Scope potassium titanyl phosphate laser at 532 nm. Laser therapy was under the direction of the staff surgeon, as was the final laser margin.

Polymerase Chain Reaction Techniques. The DNA extracts were obtained from equal-size tissue fragments measuring approximately 0.05 cm^3 in volume. Each tissue fragment was treated with 500 µL of xylene in 2 sequential washings followed by 2 sequential washings in 500 µL of absolute ethanol. The tissue was dried for approximately 3 to 8 hours with a vacuum pump at room temperature. Extracts were created with a solution of 100-mmol/L Tris and 1-mmol/L ethylenediaminetetraacetic acid with 400 µg/mL of proteinase-K and incubated at 55° for 3 hours. The extracts were boiled for 10 minutes. Total extraction volume ranged from 100 to 300 µL, depending on tissue size.

The final DNA concentration was 100 to 500 µg/mL. Five- to 10-µL aliquots were used in each polymerase chain reaction (PCR). Samples from paraffin-fixed tissue were extracted by the same method described above with 7 to 10 slices (8 µm thick each).

The PCR was performed as described by Manos et al.[7] Primers used in this study (MY09 and MY11) were designed by Manos et al to amplify a 450 base pair fragment of DNA from common types of HPV (HPV 6, 11, 16, 18, and 31/33). To ensure the presence of amplifiable DNA in negative reactions, each sample was also tested with β-globin primers designed to amplify the 350 base pair fragment from human β-globin gene. Physical separation of DNA extractions and amplification areas were provided to avoid false-positive PCR results due to amplicon carryover. The PCRs were carried out at a volume of 75 µL with a final concentration of 4-mmol/L magnesium chloride, 20 pmol of each primer, and 1.25 units of Taq-Polymerase. Reactions were overlaid with 100 µL mineral oil and subjected to 40 cycles on a Perkin-Elmer Cetus.

Dot-Blot Hybridization. Following PCR amplification, 6 µL of each PCR reaction mixture was blotted onto 6 different nylon membranes (Zeta-probe, Bio-Rad) with a dot-blot apparatus. The membranes were then hybridized to digoxigenin-labeled oligonucleotide probes that contained sequences specific for HPV, including a consensus probe and probes specific for HPV 6, HPV 11, HPV 16, HPV 18, and HPV 31/33. Following hybridization, the membranes were washed once with 2X SSPE followed by 2 washes with 0.5X SSPE. The hybridized probes were detected with an alkaline-phosphatase–conjugated antibody directed against digoxigenin followed by chemoluminescent detection with a Rad-Free system (Sarstedt).

RESULTS

Thirteen patients with recurrent papilloma of the upper aerodigestive tract were included in this study (Table 1). The age of the patients ranged from 2 to 39 years, and the duration of disease ranged from 1 to 35 years. Three of the patients exhibited adult-onset disease, while the remaining 10 cases were of pediatric onset. All patients presented with common symptoms of papilloma requiring surgical therapy.

Patients were enrolled in the study from April 1995 to December 1995. Surgery was directed by 1 of 4 staff surgeons. Patients underwent standard endoscopic laser therapy with either a potassium titanyl phosphate laser or a carbon dioxide laser. Four of the patients were also receiving adjuvant therapy consisting of acyclovir, interferon, or "cruciferous" vi-

TABLE 2. POLYMERASE CHAIN REACTION AND HYBRIDIZATION RESULTS

Patient No.	*No. of Biopsy Pairs**	*No. Positive for HPV*	*Surgeon No.*	*Laser Type*	*HPV Type*
1	5	5	1, 2	CO_2, KTP	11
2	7	7	1	CO_2	11
3	1	1	1	CO_2	6
4	4	4	1	CO_2	11
5	5	5	1	CO_2	11
6	4	4	1	CO_2, KTP	11
7	4	4	1,2	CO_2, KTP	6
8	1	1	2	KTP	6
9	2	2	2	KTP	6
10	3	3	4	CO_2	6, 31
11	2	2	3	CO_2	6
12	2	2	1	CO_2	11
13	1	1	3	CO_2	6

HPV — human papillomavirus, CO_2 — carbon dioxide, KTP — potassium titanyl phosphate.

*Before and after laser procedure.

tamin supplement. The majority of the patients had glottic or supraglottic papillomas, with 1 patient having nasal septal lesions only. All patients had actively recurring lesions during the course of therapy.

Biopsies were performed on pre-laser and post-laser frozen tissue specimens. Each specimen was tested for presence of HPV DNA by the PCR technique. A total of 41 pairs of pre-laser and post-laser specimens from the 13 patients were processed (Table 2). All (100%) post-laser specimens were positive for HPV DNA. To monitor for possible intraoperative contamination resulting in false positives, adenoid tissue from 10 patients without papilloma was tested for HPV DNA by PCR. All 10 specimens were negative for HPV DNA and were positive for human β-globin gene (data not shown).

The specimens from each patient were subtyped for the common mucosal HPV species by dot-blot hybridization techniques. A total of 7 patients were positive for HPV type 6, and 6 were positive for HPV type 11. One patient was also positive for HPV type 31.

Hematoxylin and eosin–stained sections from biopsy specimens taken before and after treatment were examined for evidence of HPV infection. Sections taken from papillomas before treatment consisted of fragments of squamous epithelium containing numerous cells with cytologic features consistent with HPV infection, including hyperchromatic raisin-shaped nuclei, perinuclear halos, and multinucleate forms. Sections taken from laryngeal mucosa after treatment consisted of coagulated submucosa and small fragments of squamous epithelium. In some of the posttreatment sections, the squamous epithelium was found to contain cells with cytologic features of HPV infection (Fig 1).

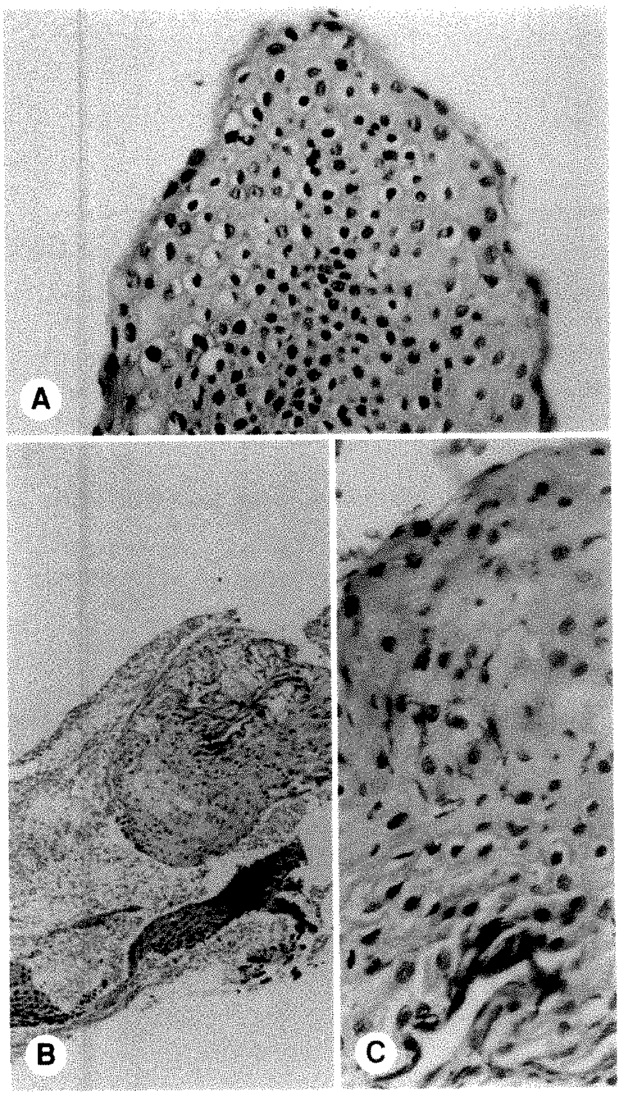

Fig 1. Biopsy specimens (H & E). A) Pre-laser (original ×75). B) Post-laser (original ×40). C) Post-laser (original ×100).

The above histologic samples were tested for the presence of HPV DNA by PCR techniques. Paired specimens were cut from paraffin blocks and DNA was extracted for PCR analysis. All specimens tested positive for HPV DNA. Results of 2 paired specimens are shown in Fig 2.

DISCUSSION

The group of patients in this study have the typical characteristics for RRP.[8] The pediatric-to-adult ratio is 3 to 1. The site of lesion is primarily laryngeal (glottic or supraglottic). All patients have required multiple surgeries for recurrent disease, and viral types were either 6 or 11, the subtypes most common in RRP.

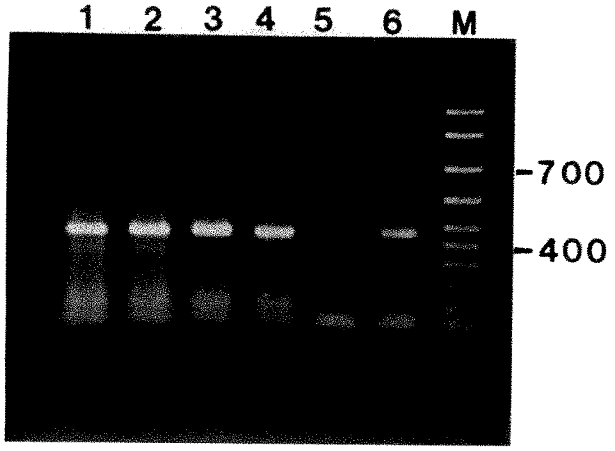

Fig 2. Gel electrophoresis of samples amplified with human papillomavirus (HPV) consensus primer (MY09 and MY11). Lanes 1-4 are tissue extracts from paraffin blocks. Lanes 1 and 2 are pre-laser and post-laser samples from 1 patient. Lanes 3 and 4 are pre-laser and post-laser samples from another patient. Lane 5 is negative control containing water only. Lane 6 is HPV plasmid control. Lane M is molecular weight marker ladder; 400 and 700 indicate base pair markers. Lanes contain equal amounts of amplification mixture.

The postulated mechanisms by which papillomas recur include infection from distant sites, stimulation of latent virus, and incomplete removal of papilloma from the surgical site. It has been demonstrated that clinically normal mucosa surrounding papilloma can harbor latent virus.[3,9] Other authors have demonstrated measurable viral DNA at locations throughout the aerodigestive tract in patients with RRP.[10] This study demonstrates that there is residual papilloma DNA following laser therapy.

Since PCR is a highly sensitive technique for detecting the presence of viral DNA, we felt that it would be the best technique for determining the presence or absence of viral DNA at the laser bed. The results of our 41 paired surgical biopsies demonstrated HPV DNA in all cases and indicate that laser therapy does not eradicate viral DNA from the papilloma laser bed. These results are in agreement with a publication involving anogenital papilloma.[11] The authors were able to identify viral DNA in papilloma lesion sites in an equal percentage before and after laser therapy. However, histologic evaluation at the time of surgery was not done.[11]

In the present study, tissue samples were taken from lesions that were visibly free of papilloma at the end of laser therapy. The histologic findings of the post-laser specimens support the results of the PCR experiments; in those specimens with intact islands of squamous epithelium, cytologic features consistent with HPV were identified. This finding indicates that microscopic papillomas remain after all gross evidence of papillomas has been vaporized.

It is apparent from these findings that the high percentage of positive PCR results is due to incomplete vaporization of native papilloma.

One concern in light of such results is the possibility of contamination during laser treatment. It has been shown that there is PCR-detectable HPV DNA in laser plume of RRP lesions.[12] However, when plume collection was carefully controlled, and contamination by intact cells was eliminated, only 64% of plumes were found to be positive for HPV. As well, the PCR signal was reported as weaker in the plume as compared to tissue samples. In our study we had 100% positive samples for PCR and very little variation in PCR signal. We attempted in situ hybridization of the post-laser tissues, but we were unable to detect measurable viral DNA (data not shown). This result can be explained by the low number of copies of viral DNA in RRP lesions,[13] particularly in the deeper portions of the lesions that would be sampled after laser therapy.

Laser therapy is subject to many variables that can affect the ability to completely excise a lesion. These include individual surgical technique, variability of the laryngeal lesions, and unpredictability of laser energy delivery. It is unlikely that the persistence of detectable viral DNA and squamous epithelium in our samples was the result of individual surgical technique. Our samples were taken under the direction of 4 different surgeons in an unbiased fashion with the exception of choosing biopsy sites with the least probability of injury to the patient. Post-laser biopsy sites were chosen from what appeared to be clear surgical margins under view of the operating microscope. The inability to completely remove papilloma with the laser stems from the difficulty in detection of small remnant papilloma that results even with the most fastidious laser coagulation. Using the canine larynx, authors have shown unpredictable distribution of laser energy in normal squamous mucosa.[14] The papilloma structure is highly irregular, with multiple fronds and fibrovascular cores making the even distribution of laser energy more difficult.

The low probability of completely removing papilloma at surgery underscores the need for adjuvant therapy. Adjuvant therapies have included hormones, antimetabolites, and photodynamic therapy and have all shown modest response.[2] The 4 patients in our group that were on adjuvant therapy showed no difference in PCR or histology results. Future strategies for adjuvant therapy should be directed at potentiating laser ablation. These approaches may include the development of new photodynamic agents that are activated at standard surgical laser wavelengths.[15]

The inability to completely remove papilloma at surgery also emphasizes the need for prudent laser therapy. There are many articles in the literature demonstrating both the cost and complications of multiple laser surgeries for RRP.[16] Canine studies have demonstrated that despite precise laser technique, much edema and destruction, as well as submucosal fibrosis of the vocal folds, can occur.[17] These complications can be minimized only with conservative laser therapy directed at palliation rather than complete removal of papilloma, particularly in areas in which damage would affect laryngeal function. More aggressive laser treatment could be reserved for areas that are functionally less important.

SUMMARY

We found viral DNA consistently present following laser therapy in RRP patients. Histologic findings indicate that remnant papilloma is likely the source of the viral DNA. The remnant papilloma likely serves as a nidus for recurrent papilloma growth and may explain RRP recalcitrance to conventional laser therapy.

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REFERENCES

1. Kashima HK. Recurrent respiratory papillomatosis. In: Gates GA, ed. Current therapy in otolaryngology–head and neck surgery. 5th ed. St Louis, Mo: Mosby–Year Book, 1994:441-3.
2. Avidano MA, Singleton GT. Adjuvant drug strategies in the treatment of recurrent respiratory papillomatosis. Otolaryngol Head Neck Surg 1995;112:197-202.
3. Kashima H, Mounts P, Leventhal B, Hruban RH. Sites of predilection in recurrent respiratory papillomatosis. Ann Otol Rhinol Laryngol 1993;102:580-3.
4. Braun L, Kashima HK, Eggleston J, Shah K. Demonstration of papillomavirus antigen in paraffin sections of laryngeal papillomas. Laryngoscope 1982;92:640-3.
5. Gissmann L, Diehl V, Schultz-Coulon H-J, Zur-Hausen H. Molecular cloning and characterization of human papilloma virus DNA derived from a laryngeal papilloma. J Virol 1982;44: 393-400.
6. Steinberg BM, Topp WC, Schneider PS, Abramson AL. Laryngeal papillomavirus infection during clinical remission. N Engl J Med 1983;308:1261-4.
7. Manos MM, Ting Y, Wright DK, Lewis AJ, Broker TR, Wolinsky SM. Use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. In: Furth M, Greaves M, eds. Cancer cells. Vol 7. Cold Spring Harbor, NY: Cold Spring Harbor Press, 1989:209-14.
8. Benjamin B, Parsons D. Recurrent respiratory papillomatosis: a 10 year study. J Laryngol Otol 1988;102:1022-8.
9. Abramson AL, Steinberg BM, Winkler B. Laryngeal papillomatosis: clinical, histopathologic and molecular studies. Laryngoscope 1987;97:678-85.
10. Smith EM, Pignatari SS, Gray SD, et al. Human papillomavirus infection in papilloma and nondiseased respiratory sites of patients with recurrent respiratory papillomatosis using the polymerase chain reaction. Arch Otolaryngol Head Neck Surg 1993;119:554-7.
11. Lassus J, Happonen H-P, Niemi K-M, Rank A. Carbon dioxide (CO_2)–laser therapy cures macroscopic lesions, but viral genome is not eradicated in men with therapy-resistant HPV infection. Sex Transm Dis 1994;21:297-302.
12. Kashima HK, Kessis T, Mounts P, Shah K. Polymerase chain reaction identification of human papilloma virus DNA in CO_2 laser plume from recurrent respiratory papilloma. Otolaryngol Head Neck Surg 1991;104:191-5.
13. Abramson AL, DiLorenzo TP, Steinberg BM. Is papillomavirus detectable in the plume of laser-treated laryngeal papilloma? Arch Otolaryngol Head Neck Surg 1990;116:604-7.
14. Stern LS, Abramson AL, Grimes GW. Qualitative and morphometric evaluation of vocal cord lesions produced by the carbon dioxide laser. Laryngoscope 1980;90:792-808.
15. Abramson AL, Shikowitz MJ, Mullooly VM, Steinberg BM, Amell CA, Rothstein HR. Clinical effects of photodynamic therapy on recurrent laryngeal papillomas. Arch Otolaryngol Head Neck Surg 1992;118:25-30.
16. Saleh EM. Complications of treatment of recurrent laryngeal papillomatosis with the carbon dioxide laser in children. J Laryngol Otol 1992;106:715-8.
17. Durkin GE, Duncavage JA, Toohill JR, Tieu TM, Caya JG. Wound healing of true vocal cord squamous epithelium after CO_2 laser ablation and cup forceps stripping. Otolaryngol Head Neck Surg 1986;95:273-7.

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EFFECT OF PHARMACOLOGICAL SYMPATHECTOMY ON OSTEOCLASTIC ACTIVITY IN THE GERBILLINE AUDITORY BULLA IN VIVO

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Bone destruction causes hearing loss in various middle ear disorders. The mechanisms of such pathological remodeling are unknown. Unilateral surgical sympathectomy is known to induce resorption within mandibular and auditory bulla bone. Explanation of the cause of this effect, however, may be confounded by hemodynamic changes induced by hemicranial sympathectomy and by uncertainty as to the neuroanatomical origins of sympathetic fibers. In this study, gerbils were infused with guanethidine sulfate (GS) to evaluate the in vivo effects of systemic sympatholysis on auditory bone remodeling. In addition, to discount any direct osteolytic effect, GS was assessed of its bone resorbing activity in vitro by means of the calvarial calcium release assay. The in vitro study revealed GS to have no effect on calcium release. The in vivo study revealed GS to increase both the osteoclast surface and number. Guanethidine-induced sympathectomy has thus been shown to increase remodeling in gerbilline auditory bone, while no direct osteolytic effect could be measured in vitro.

KEY WORDS — bone resorption, guanethidine sulfate, pressure, sympathectomy.

INTRODUCTION

Localized bone modeling and remodeling are the processes that lead to the pathological effects of several important ear diseases such as chronic otitis media, otosclerosis, osteopetrosis, Paget's disease of bone, and osteogenesis imperfecta.[1] A number of factors have been implicated in these processes, such as prostaglandins,[2-4] leukotrienes,[5,6] interleukins,[7-12] tumor necrosis factor,[9,11,13] interferon,[7,8,14] transforming growth factors,[15-17] and physical strain.[18-21] In this study, we evaluated the effect of pharmacological sympathectomy with guanethidine sulfate (GS) on middle ear bone remodeling. Guanethidine sulfate is a selective sympatholytic agent that has antihypertensive activity. In experiments using neonatal and adult rats, the agent has been shown to reduce tissue catecholamine levels, eliminate tyrosine hydroxylase immunoreactivity, and destroy nerve cell bodies within sympathetic ganglia.[22-24] Interestingly, these effects are thought to be mediated not through direct cytotoxic effects, but via a drug-induced, cell-mediated, autoimmune mechanism specific to the rat.[25,26]

Because the sympatholytic effects of GS are well established in the rat, the agent has been used extensively to evaluate the relationship between sympathectomy and bone metabolism in that model. Those studies have shown GS to significantly decrease the uptake of [^{3}H]-proline by osteoblasts of the rat molar root socket.[27,28] It has also been shown to significantly decrease the osteoblastic uptake of [^{3}H]-proline in bone of the rat femoral diaphysis.[29] In contrast, the surface of rat mandibular bone, but not tibial long bone, has been shown to undergo a 45% increase in osteoclast number after neonatal sympathectomy with GS.[30] These results indicate that in the rat, GS-induced sympathectomy stimulates osteoclastic activity within mandibular bone, while it inhibits osteoblastic activity in both mandibular and long bone. In animal models other than the rat, however, data regarding the effects of GS-induced sympathectomy on bone remodeling are unavailable.

In previous studies, we have shown surgical sympathectomy (induced by the unilateral resection of ascending sympathetic fibers) to significantly increase the osteoclast number and the osteoclast surface of gerbilline auditory bulla bone when compared to control bone harvested from contralateral, sham-operated bullae.[31] Data from that experiment supported the hypothesis that sympathectomy increases resorption in membranous bone. The present study was therefore proposed to confirm the resorptive effects of sympathectomy in the gerbil with an entirely different mode of treatment: systemic pharmacological sympathectomy with continuously infused GS.

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The intent of this systemic approach is to eliminate the potential effect of regional hemodynamic differences induced by hemicranial surgical sympathectomy, to avoid the uncertain anatomic origins of the bulla bone's sympathetic fibers, and to circumvent the inability of surgical sympathectomy to completely eliminate postganglionic fibers. The present experiment also utilizes middle ear air pressure as a positive control for osteoclastic induction — a control not utilized in our previous experiment, and our laboratory's standard for localized osteoclastic induction in the gerbilline middle ear.[18] Further, this investigation evaluated the effects of GS on membranous bone resorption in vitro with the calvarial organ culture calcium release assay. This assessment was critical, because if GS was found to exert a direct effect on target bone independent of its effects on the sympathetic nervous system, then the cause-and-effect assertion of "sympathectomy-induced resorption" would be undermined. Hence, the direct effects of GS on membranous bone metabolism were determined in vitro prior to the evaluation of GS's sympathectomy-induced effects on gerbilline middle ear bone resorption in vivo.

METHODS AND MATERIALS

IN VITRO STUDY

Experimental Design. The effects of GS, at both 10^{-8} mol/L and at 10^{-6} mol/L concentrations, were determined on 72-hour calcium release from cultured neonate mouse hemicalvaria. The substance was assessed for its activity with and without parathyroid hormone (PTH): with PTH, the ability to inhibit PTH-stimulated calcium release was determined, while without PTH, the ability to induce calcium release from unstimulated bone was determined. Each treatment condition was assessed with 8 neonate mouse hemicalvarial explants cultured in serum-enriched medium. After a 24-hour preincubation period, the bone explants were cultured for an experimental period of 72 hours, at the end of which samples from the treatment and control media were analyzed for calcium concentration. The ability of a treatment to either induce bone resorption or inhibit PTH-stimulated bone resorption was thus quantified as the net change in the incubation medium's calcium level when compared to controls.

Animal Subjects. Pregnant Swiss-Webster mice, timed to deliver 3 days prior to each study, were obtained from Simonsen Laboratories (Gilroy, Calif). Their use was approved by the Institutional Animal Care and Use Committee of the University of California Davis (IACUC protocol 4676). The animals were placed in individual cages housed in a temperature-controlled animal room maintained on a 12-hour light-dark cycle. Approximately 3 days after parturition, the weight of each neonate was recorded and the animals were placed into groups of 8. The groups were further arranged to minimize differences in the means and distributions of weights, and animals weighing greater than 1 g above or below the mean were eliminated.

Guanethidine. The sympatholytic agent GS ([2-(hexahydro-1(2H)-azocinyl)-ethyl] guanidine sulfate) was donated by Ciba Geigy (Summit, NJ) and used without further purification. The substance was dissolved in sterile culture water and refrigerated at 4°C. Two stock solutions of GS were prepared so that a 10 µL aliquot added to 2 mL of medium would result in a final concentration of 10^{-6} or 10^{-8} mol/L.

Incubation Medium. Four milliliters of incubation medium were required for each hemicalvarium: 2 mL for preincubation and 2 mL for the experimental period. The stock medium consisted of 15% heat-inactivated horse serum (No. H7889, lot 49F-0082, Sigma Chemical Co, St Louis, Mo), 84% Dulbecco's modified Eagle's medium (No. 320-1965 AG, Gibco Laboratories, Grand Island, NY), and 1% antibiotic-antimycotic in 0.9% saline (No. A7292, lot 119F-4606, Sigma). The horse serum was first heat-inactivated for 1 hour in a constantly stirred 59°C water bath. The lyophilized antibiotic was reconstituted and dispensed at 10 µL/mL of incubation medium to give final medium concentrations of 100 U/mL of penicillin G potassium, 0.1 mg/mL of streptomycin sulfate, and 0.25 µg/mL of amphotericin B sodium deoxycholate.

The effect of GS was evaluated with and without the addition of PTH. Synthetic bovine PTH (lot 11H48301, Sigma) was used without additional purification. The hormone was reconstituted in sterile culture water and refrigerated at 4°C. After accounting for the purity of the sample lot, the concentration of the stock solution was calculated so that 10 µL added to 2 mL of incubation medium would result in a final medium concentration of 10^{-8} mol/L.

Sets of 10 sterile Pyrex test tubes were filled with base medium and GS with or without PTH. The 9th and 10th tubes of each set were refrigerated prior to incubation without the addition of bone so that a baseline (preincubation) calcium level could be measured. This preincubation baseline calcium level, determined from the mean calcium level of the medium of tubes 9 and 10, was then subtracted from the final postincubation calcium level of tubes 1 through 8 to determine the net calcium release value of each experimental tube.

Calvarial Culture. The 8 neonatal mice of each group were euthanized in a carbon dioxide chamber

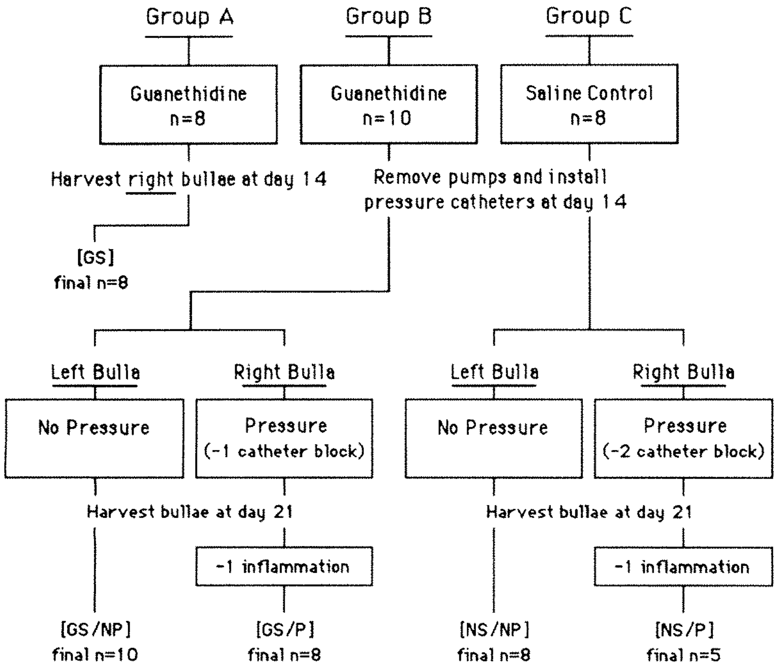

Fig 1. Diagram of investigational treatment arms for in vivo experiment. Each animal underwent 2 weeks of either saline infusion (control) or guanethidine sulfate (GS) infusion (experimental). Animals of group A were euthanized after 14-day treatment period to determine 2-week, baseline effect of GS on right middle ear bone resorption. In contrast, animals of groups B and C had their infusion pumps removed and underwent additional 7 days of unilateral pressurization of their right middle ear bullae. These animals were euthanized at day 21, and their left and right bullae were harvested for histomorphometric assessment. NP — no pressure, P — pressure, NS — no sympathectomy.

and the bodies reweighed and submerged in 70% ethanol for 3 minutes. After removal from the alcohol, the mice were air-dried under a sterile hood, and their frontoparietal calvaria were dissected, trimmed, and split into halves. Each bone explant was immediately placed into an appropriately labeled sterile test tube containing 2 mL of freshly prepared medium and gassed with a mixture of 50% oxygen, 45% nitrogen, and 5% carbon dioxide (Liquid Carbonic Co, Chicago, Ill). The tubes were then stoppered and placed in a rotating drum (Bellco Glass Inc, Vineland, NJ) inclined 5° above the horizontal axis and rotating at 1 rpm. Finally, the roller drum was placed in a humidity-controlled culture oven maintained at 37.5°C.

After an initial 24-hour stabilization period, the medium of each set of 8 tubes was discarded and replaced (2 mL per tube) with stock medium plus GS with or without PTH. Stock GS solution was added directly to the tubes in 10 µL aliquots to give final concentrations of 10^{-6} or 10^{-8} mol/L. The tubes with hemicalvaria and fresh medium were then gassed and incubated at 37.5°C for 72 hours, with gassing every 24 hours. Last, each group's baseline calcium control sample tubes (the 9th and 10th tubes of each group, which did not receive a bone explant) were filled with 2 mL of the identical medium used in the preceding 8 incubation tubes and refrigerated.

Calcium Analysis. The concentration of calcium (in micrograms per milliliter) was determined with a Perkin Elmer 3030 B atomic absorption spectrophotometer. Three samples from each experimental or control tube were analyzed to give a mean calcium content per tube. The mean values for control tubes 9 and 10 were further averaged to yield that group's mean preincubation calcium value. This value was then subtracted from the mean calcium release values of the experimental tubes to determine their net calcium release.

The experimental and control calcium values were compared as unmatched groups. Comparisons were conducted via one-way analysis of variance with Bonferroni-corrected multiple comparisons post hoc tests (ie, between-group analysis) on the InStat 2.0 biostatistical program (GraphPad Software, San Diego, Calif). Significance for each comparison was set at the 95% level.

IN VIVO STUDY

Experimental Design. Three treatment groups were used to provide combinations of unilateral middle ear pressurization and continuous subcutaneous infusion of either vehicle (normal saline) or GS via osmotic minipumps. Twenty-six male Mongolian gerbils were arranged into 3 groups: A (n = 8), B (n = 10), and C (n = 8; Fig 1). The animals of groups A and B received GS at 20 mg/kg per day for 14 days via subcutaneously implanted osmotic minipumps (Alzet Corporation, Palo Alto, Calif), while the animals of group C received saline via identical pumps to serve as the unsympathectomized, unmatched controls. The success of drug delivery and activity was determined from the appearance of marked bilateral ptosis in the GS-infused animals 1 day following minipump implantation.

On the 14th treatment day, the group A animals were euthanized and bone samples from their right middle ear bullae were harvested and fixed. This allowed determination of a 2-week baseline effect of GS treatment on bulla bone resorption. At the same time, the minipumps of the group B and C animals were removed, and their bullae were implanted with stainless steel catheters for the unilateral pressurization of their right middle ear bullae. The left bullae of these animals were similarly implanted with pairs of sham catheters to serve as the right bullae's unpressurized, matched controls. The animals were then placed in circular cages in which their pressure catheters could be attached to swiveled, overhead air pressure outlets delivering 10 mm Hg of positive air pressure to each right bulla.[18] The system thus allowed each group B and C animal a free range of motion while specifically attaching their inflow catheters to a common adjustable air line. After 7 days of continuous right bulla pressurization (day 21), the animals of groups B and C were euthanized and bone samples from their right and left middle ear bullae were harvested. Each bone sample was then fixed, sectioned, and analyzed histomorphometrically to determine the osteoclast number (N.Oc/T.L), the osteoclast surface (Oc.S/BS), and the individual osteoclast profile area (Oc.Profile). This combination of treatments thus allowed for the quantification of the effects of guanethidine sympathectomy on osteoclast-mediated middle ear bone resorption, both alone and on bone simultaneously activated by positive air pressure (ie, adaptive bone remodeling; Fig 1).

Animal Subjects. Twenty-six outbred male Mongolian gerbils (*Meriones unguiculatus*), averaging 90 to 110 days old and 64 g in weight, were used in the experiment. The animals were purchased from Tumblebrook Farms (West Brookfield, Mass) and placed in individually numbered circular cages mounted on transportable upright racks. The cage racks were housed in a temperature-controlled animal room maintained on a constant 12-hour light-dark cycle. Each animal was given constant access to rodent chow and water. Approval for the animals' care and use was obtained from the Institutional Animal Care and Use Committee of the University of California Davis (IACUC protocol 4012), and procedures conformed to all Public Health Service and National Institutes of Health policies on the humane treatment of laboratory animals.

Pharmacological Sympathectomy. Subcutaneously implanted Alzet model 2002 osmotic minipumps capable of delivering solutions at 0.5 μL/h for 14 days were used to continuously infuse GS or vehicle (normal saline). Eighteen adult male gerbils randomly designated "experimental" were implanted with pumps filled with GS (Ciba Geigy), while 8 animals designated "control" received pumps loaded with vehicle only (0.9% sterile saline) to serve as unsympathectomized, unmatched controls. Each pump was weighed before and after filling to ensure complete loading, and all procedures were conducted with sterile technique. The concentration of GS (93.39 mg/mL) was determined so that each pump would deliver 20 mg/kg per day during the 14-day treatment period.

The minipumps were implanted while the animals were under continuous 2,2dichloro-1,1-difluoroethylmethylether (Metofane) anesthesia (Pittman-Moore, Mundelein, Ill). After the region between the scapulae of each gerbil was shaved and the underlying skin was prepared for sterile surgery, a 1.5 cm transverse incision was made through which a small subdermal pocket was created. The minipumps were inserted aperture-first into the pockets, and the incisions were closed with sterile 4-0 silk sutures. The animals were then placed in warmed cages and continuously monitored until they were ambulatory. The success of drug delivery and activity was determined from the appearance of marked bilateral ptosis in the GS-infused animals within 24 hours of pump implantation.

Middle Ear Pressurization. With the gerbilline skull used as a full-scale model, matching pairs of stainless steel inflow and outflow catheters were manufactured from 18-gauge, 1.5 inch stainless steel hypodermic needles (Monoject, St Louis, Mo) and shaped to the ventrolateral aspect of the otic capsule. Blunt sham tubes for implantation into the unpressurized left bullae were similarly machined and shaped. The fur overlying each gerbil's dorsal cranium was shaved and prepared for sterile surgery. A transverse incision was made between the pinnae, exposing the dorsum of the skull. A diamond-tipped, high-speed dental drill was used to make two 1.2 mm diameter holes in the exposed aspect of each bulla. Two additional holes were also drilled into the exposed dorsomedial calvarium to allow placement of two No. 0-80, $^1/_8$ inch anchoring screws (Small Parts Inc, Miami, Fla).

The previously machined inflow and outflow catheters were fitted to the 2 holes drilled into each animal's right bulla. Pairs of sham catheters were similarly fitted to the remaining 2 holes in the left bullae to serve as the unpressurized, matched controls. All of the components were gently repositioned for optimal fit and secured to the skull with dental acrylic (Grip Cement, R & R Dentsply, Maumee, Ohio).

After implantation of the pressure catheters and complete hardening of the imbedding cement, but prior to each animal's complete anesthetic recovery, 2 segments of PE-160 polyethylene tubing (Intramedic, Clay Adams, Parsippany, NY) were fitted over the ends of the paired inflow and outflow catheters emerging from the dental cement. A custom-made, stainless steel spring (3.0 mm outside diameter by 30 cm; Small Parts Inc) was then slipped over these tubes to provide rigidity and to serve as a protective casing. Fresh acrylic was next applied to cement the catheter-tube connections and further secure the base of each spring to the pressure headmounts.

Once the acrylic had hardened and the animals had fully recovered, the gerbils were returned to their individual cages, where their pressure tubings could be extended and attached to swiveled units mounted over the tops of their cages. Line pressure was supplied by an electric diaphragm pump routed through two 5 μL in-line Acrodisc filters (Gelman Scientific, Ann Arbor, Mich). Central pressure was adjusted to provide a constant 10 mm Hg of positive pressure to each right bulla via the headset's inflow tube. The matching outflow tubes (in the pressurized bullae) were blocked by removable plugs to allow measurement of actual pressures and to ensure patency. Adequacy of pressurization was checked twice daily by demonstrating that a steady stream of air bubbles came from the tip of each outflow catheter when the catheter was submerged in water. Failure to demonstrate airflow eliminated the subject animal from the study.

Tissue Processing and Analysis. On day 14 for group A, and on day 21 for groups B and C, the animals were euthanized via asphyxiation in a carbon dioxide chamber. The ventral wall of each animal's middle ear bulla was exposed and a 5 × 2 mm bone sample was resected, leaving the inner and outer periosteum intact. The samples were then fixed in 2% glutaraldehyde and 2% paraformaldehyde in 0.1 mol/L cacodylate buffer at pH 7.4 at 3°C. After fixation, the specimens were decalcified in 0.1 mol/L tetrasodium ethylenediaminetetraacetic acid with 4% glutaraldehyde and trimmed to leave a 4 × 2 mm section of ventral bulla wall. The samples were then dehydrated in graded solutions of acetone, embedded in Epon-araldite, and sectioned to 1 μm on a Reichert ultramicrotome. Each bulla bone section was taken along the sample's greatest length (ie, along the skull base anteroposterior axis).

Three sections, spaced at least 100 μm apart, were taken from each bone sample. The sections were stained with toluidine blue and basic fuchsin, blind-labeled, and evaluated histomorphometrically with an Olympus BH2 light microscope (Olympus Optical Co, Tokyo, Japan). Osteoclasts were identified as multinucleate cells with ruffled borders abutting bone. Only the inner or concave surface of each sample was analyzed. Measurements of length of bone surface, length of bone occupied by osteoclasts, and mean osteoclast profile sizes were obtained from camera lucida tracings using the VIAS computer-based histomorphometry system (Ted Pella, Redding, Calif).

Three factors were quantified from the raw data in the standardized units of bone histomorphometry.[32] They were 1) the number of osteoclasts per linear millimeter of bone surface, termed osteoclast number, denoted as N.Oc/T.L and expressed in number per millimeter; 2) the percent of bone surface lined by osteoclasts, termed osteoclast surface, denoted as Oc.S/BS and expressed as a percentage; and 3) the mean osteoclast area, termed osteoclast profile, denoted as Oc.Profile and expressed in square micrometers.

Three specimens were analyzed from each bone sample, and the entire length of each specimen was measured. Final data were obtained by averaging the measurements taken from each sample's triplicate sections. Statistical analyses of the data were then conducted with appropriate paired or unpaired two-tailed Student's *t*-tests or one-way analysis of variance with Bonferroni-corrected multiple comparisons post hoc tests (InStat 2.0, GraphPad Software). For all comparisons, significance was determined at the 95% level.

RESULTS

IN VITRO STUDY

The activity of GS on net 72-hour calcium release was determined on murine hemicalvaria incubated with or without PTH. Guanethidine sulfate, both at 10^{-8} and at 10^{-6} mol/L, was found to have no stimulatory effect on calcium release from bone when compared to bone incubated in base media alone ($p > .05$). Similarly, GS at 10^{-8} and 10^{-6} mol/L exerted no significant inhibitory effect on PTH-stimulated calcium release when compared to PTH-stimulated controls ($p > .05$; Table 1).

IN VIVO STUDY

All 26 gerbils implanted with subcutaneous infusion pumps completed the 2-week treatment period. In animals implanted with GS-containing pumps, a marked bilateral ptosis was displayed within 12 hours of implantation and continued throughout the treatment period. No other gross changes were observed associated with the treatment regimen, which was apparently well tolerated by all subjects.

On the 14th treatment day, the GS-treated animals of group A were euthanized and bone samples from their right bullae were harvested. At the same time, pumps from the group B and C animals were re-

TABLE 1. RESULTS OF IN VITRO EXPERIMENTS COMPARING EFFECTS OF GS WITH AND WITHOUT PTH ON 72-HOUR CALCIUM RELEASE FROM NEONATE MOUSE HEMICALVARIA

					Comparisons	
	Group	*Condition*	*N*	*72-Hour Calcium Release (μg/mL)*	*Versus Negative Control*	*Versus Positive Control*
Treatments	1	GS 10^{-8} mol/L	8	21.1 ± 9.5	$p > .05$	$p < .001$
	2	GS 10^{-6} mol/L	8	10.1 ± 13.8	$p > .05$	$p < .001$
	3	GS 10^{-8} mol/L and PTH 10^{-8} mol/L	8	34.4 ± 10.0	$p < .001$	$p > .05$
	4	GS 10^{-6} mol/L and PTH 10^{-8} mol/L	8	53.2 ± 18.7	$p < .001$	$p > .05$
Controls	5	Saline (negative control)	8	10.2 ± 8.3		$p < .001$
	6	PTH 10^{-8} mol/L (positive control)	8	49.3 ± 5.6	$p < .001$	
					$F_{5,42} = 20.782$ $p < .0001$	

One-way analysis of variance with Bonferroni-corrected post hoc tests was used to compare measurements. Negative control is normal saline (group 5). Positive control is parathyroid hormone (PTH) at 10^{-8} mol/L (group 6). Note that guanethidine sulfate (GS), both at 10^{-8} and 10^{-6} mol/L, had no effect on unstimulated and PTH-stimulated calcium release, while PTH at 10^{-8} mol/L increased calcium release regardless of GS presence.

moved and the animals were implanted with right bulla pressure catheters and left sham catheters, respectively. During the subsequent 7-day pressurization period, 1 bulla from group B and 2 from group C were eliminated from their treatment groups due to irreparable catheter blockage. On the 21st day, the remaining animals were euthanized and bone samples from their right and left bullae were harvested and fixed. At the time of death, the bilateral ptosis displayed throughout the infusion period was still present, although to various degrees, in all remaining group B animals. Further, 2 additional bullae, 1 each from group B and group C, were eliminated from the study due to gross middle ear inflammation discovered during bone harvesting.

After the bone samples from the 3 treatment groups were analyzed, data regarding osteoclast number, osteoclast surface, and osteoclast profile were determined. The resulting 5 conditions were thus: 1) Guanethidine Sympathectomy for 14 days only ([GS], n = 8); 2) Guanethidine Sympathectomy for 14 days/No Pressure for 7 days ([GS/NP], n = 10); 3) Guanethidine Sympathectomy for 14 days/Pressure for 7 days ([GS/P], n = 8); 4) No Sympathectomy (saline) for 14 days/No Pressure for 7 days ([NS/NP], n = 8); and 5) No Sympathectomy (saline) for 14 days/Pressure for 7 days ([NS/P], n = 5).

Effect of Pressure on Bone Resorption. Bone samples harvested from the group C animals provided the paired pressurized versus unpressurized conditions of [NS/P] and [NS/NP], respectively. The osteoclast number of bone from the pressurized bullae ([NS/P]) was significantly greater than that of bone from the unpressurized, contralateral controls ([NS/NP]; p = .005). The osteoclast surface of bone from the pressurized bullae ([NS/P]) was also significantly greater than that of the sham-operated controls ([NS/NP]; p = .002). Likewise, an increase in the mean osteoclast profile of bone was observed in the pressurized bullae ([NS/P]) when compared to the unpressurized, contralateral controls ([NS/NP]; p = .025; Table 2).

TABLE 2. SUMMARY OF OSTEOCLASTIC MEASUREMENTS OBTAINED FROM FIVE GROUPS OF IN VIVO INVESTIGATION

			Osteoclastic Measurement		
	Group	*Condition and Final Subject Number*	*Osteoclast Number (N.Oc/T.L)*	*Osteoclast Surface (Oc.S/BS)*	*Osteoclast Profile (Oc.Profile)*
Treatments	1	GS sympathectomy alone ([GS]; final n = 8)	2.4 ± 0.8 mm^{-1}	11.8% ± 4.8%	225 ± 42 μm^2
	2	GS sympathectomy, no pressure ([GS/NP]; final n = 10)	2.3 ± 0.9 mm^{-1}	11.7% ± 5.6%	242 ± 74 μm^2
	3	GS sympathectomy, pressure ([GS/P]; final n = 8)	2.6 ± 1.1 mm^{-1}	16.1% ± 8.5%	476 ± 106 μm^2
Controls	4	No sympathectomy, no pressure ([NS/NP]; final n = 8)	1.1 ± 0.4 mm^{-1}	4.8% ± 1.5%	220 ± 54 μm^2
	5	No sympathectomy, pressure ([NS/P]; final n = 5)	2.5 ± 1.0 mm^{-1}	15.3% ± 4.9%	466 ± 99 μm^2
F-value and significance for column means			$F_{4,34} = 4.0769$ $p = .008$	$F_{4,34} = 4.8256$ $p = .0034$	$F_{4,34} = 12.719$ $p < .0001$

One-way analysis of variance with Bonferroni-corrected post hoc tests was used to compare measurements. Negative control is normal saline without pressure ([NS/NP]; group 4). Positive control is normal saline with pressure ([NS/P]; group 5).

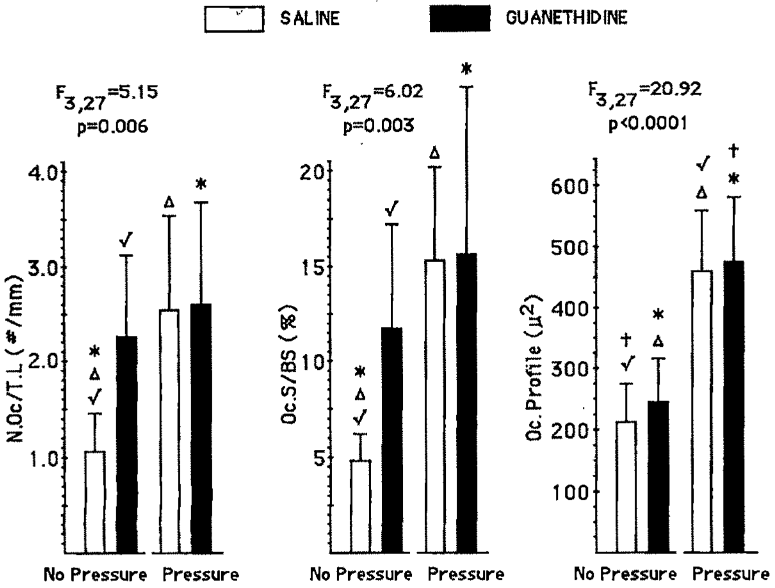

Fig 2. Results of in vivo experiment, comparing mean of each of study's 21-day treatment groups ([NS/NP], [NS/P], [GS/NP], and [GS/P]). Multiple comparisons were made between each condition via one-way analysis of variance with Bonferroni-corrected post hoc tests. Results show both sympathectomy and pressure were capable of increasing osteoclast number (N.Oc/T.L) and osteoclast surface (Oc.S/BS) of bone when compared to unpressurized, saline-infused controls. There is no evidence of interaction or summation of individual effects. In contrast, only pressure was capable of significantly increasing osteoclast profile (Oc. Profile) of bone when compared to unpressurized controls, regardless of whether bone was harvested from animals infused with saline or GS. Error bars represent standard deviations of means.

Effect of Guanethidine on Unpressurized Bone Resorption. An analysis of variance was performed on the 3 unmatched condition groups that did not receive pressure: [GS], [GS/NP], and [NS/NP]. The results show the osteoclast number of bone from the [GS] and [GS/NP] groups was significantly greater than that of unpaired bone from the saline-infused control group ([NS/NP]; p = .003). The osteoclast surface of bone harvested from the [GS] and [GS/NP] groups was also found to be significantly greater than that of the saline-infused control ([NS/NP]; p = .005). In contrast, the mean osteoclast profile was found to be no different between the control group ([NS/NP]) and the 2 GS-infused groups ([GS] and [GS/NP]; p > .05; Table 2).

Effect of Guanethidine on Pressure-Induced Bone Resorption. Bone samples from the GS-treated animals of group B provided the paired pressurized versus unpressurized conditions of [GS/P] and [GS/NP], respectively. Paired analysis of the samples revealed no difference in the osteoclast number and osteoclast surface between the GS-treated bone from pressurized bullae ([GS/P]) versus GS-treated bone from unpressurized bullae ([GS/NP]; p > .05). In contrast, the mean osteoclast profile of pressurized bone treated with GS ([GS/P]) was found to be significantly greater than that of GS-treated bone harvested from unpressurized bullae ([GS/NP]; p = .004; Table 2). Treatment with GS, therefore, eliminated the difference in osteoclast number and osteoclast surface between pressurized and unpressurized bulla bone by increasing resorption in the unpressurized bullae. In contrast, the increase in osteoclast profile induced by pressure was unaffected by concomitant treatment with GS.

An alternative assessment of the interaction of pressure and GS on bone resorption was conducted by analysis of variance. The analysis compared the 4 treatment group conditions ([GS/NP], [GS/P], [NS/NP], and [NS/P]), and hence reanalyzes the entire data set. The findings are in agreement with the paired "within-group" and unpaired "between-group" comparisons previously made. Treatment with GS alone ([GS/NP]), pressure alone ([NS/P]), or GS and pressure ([GS/P]) significantly increased the osteoclast number and osteoclast surface of bulla bone when compared to unpressurized, saline-treated controls ([NS/NP]; p < .05; Table 2 and Fig 2). No quantitative difference was found between induction by GS versus induction by pressure, and no addition or interaction between these modes was suggested. In contrast, only pressure increased the osteoclast profile of bulla bone, and no interactive effect of GS on pressure's profile-inductive effects could be seen.

DISCUSSION

This study confirmed the relationship between sympathectomy and gerbilline middle ear bone resorption by means of systemically infused GS. The study also showed GS to have no direct stimulatory effect on calcium release from cultured mouse hemicalvaria, thus supporting the cause-and-effect assertion of "sympathectomy-induced bone resorption."

The use of a systemic agent to induce sympathectomy was employed to address key issues left unresolved from our previous investigation that relied on surgical sympathectomy to induce bone resorption.[31] Those issues were 1) that the precise anatomy of the target bone's sympathetic innervation was unknown; 2) that the influence on resorption of an intact postganglionic system after preganglionic sympathectomy was unknown; 3) that the influence of vascular pressure differences between sympathectomized and unsympathectomized hemicrania was unknown; and 4) that a positive control with historical relevance was not employed. In addition, the experiment sought to determine whether GS, a sympatholytic agent whose mechanism is apparently autoimmune-dependent and rat-specific,[25,26] would induce resorption in the gerbilline model.

Systemic administration of GS was thus shown to significantly increase both the osteoclast number and the osteoclast surface of gerbilline middle ear bone, without an effect on mean osteoclast profile (Table 2 and Fig 2). These effects were fully expressed by the end of the 2-week infusion period ([GS] > [NS/NP]) and were virtually unchanged at day 21, ie, 7 days after removal of the subdermal infusion pumps ([GS/NP] = [GS]). The results mirror those obtained via surgical sympathectomy (eg, increased osteoclast number and osteoclast surface without change in osteoclast profile), and further support the relationship between sympathectomy and increased membranous bone resorption in the gerbilline middle ear.

While little is known of the effects of GS in the gerbil, GS has been extensively investigated in the rat. Adult rats treated with GS as neonates experience markedly reduced tissue norepinephrine levels and near-complete loss of peripheral tyrosine hydroxylase immunoreactivity as compared to untreated controls.[22] Comparable treatment of adult rats revealed similar elimination of catecholamine histofluorescence, in addition to the loss of greater than 95% of the nerve cell bodies of the superior cervical ganglion.[23,24] Examination of the affected ganglia via electron microscopy revealed striking alterations within neuronal mitochondria, even after only 2 weeks of treatment.[23] Moreover, histologic evaluation of GS-treated animals 1 year later showed the ganglia to have remained nearly devoid of nerve cell bodies, although the few cell bodies that survived had apparently increased in size and sprouted limited numbers of new, histofluorescent fibers.[24]

Treatment of neonate and adult rats with GS has thus been reported to 1) eliminate catecholamine histofluorescence, 2) eliminate immunoreactivity for tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, and 3) permanently destroy nerve cell bodies within rat autonomic ganglia. Investigations have shown these effects to be mediated not through direct cytotoxic effects, but via a poorly understood autoimmune mechanism that is apparently quite specific to the rat. This conclusion was made when γ-radiation, which destroys immune competence, was found to protect rats against the effects of GS treatment in a dose-dependent manner freely reversible by adoptive transfer of immune-competent cells.[25] Subsequent ultrastructural and immunohistologic characterization of this destruction showed the response to be fully initiated by the third day of GS treatment, with robust infiltration of ganglionic parenchyma by CD-8–positive, OX-19–negative lymphocytes consistent with cells of the natural killer class.[26] The studies thus suggest that GS-induced sympathectomy in the rat is a unique example of drug-induced, cell-mediated, neurolytic autoimmunity. Therefore, the effects of GS on mineralized tissue, at least in the rat, may be viewed in terms of the drug's experimentally verified sympatholytic activity.

In contrast to the rat, no detailed studies have been conducted examining the effects of GS on the gerbilline adrenergic nervous system. Nonetheless, a reference to unpublished data by Johnson and Manning[33] describes "dramatic vacuolation" of neurons without gross neuronal destruction in gerbils treated with high-dose GS. Their data suggested that GS is transported with high specificity into sympathetic neurons, causing functional loss and injury in various animal models, including the gerbil, as demonstrated by norepinephrine depletion and ultrastructural changes (ie, mitochondrial swelling and chromatolysis). However, an additional component, unique to the rat, is apparently required for the induction of irreversible ganglionic destruction via drug-induced, cell-mediated autoimmunity. No reports detailing the effects of GS on gerbilline bone metabolism can be found in the literature.

Pressure Induction and Interaction With Guanethidine. The inductive activity of unilateral pressure was determined so that the effect of GS could be compared to that of a known stimulator of osteoclastic resorption. Hence, animals infused with vehicle only provided matching pressurized versus sham-operated middle ear bones. These comparisons revealed that pressure in saline-infused animals significantly increased the osteoclast number, the osteoclast surface, and the osteoclast profile of bone as compared to bone harvested from unpressurized, saline-infused control bullae ([NS/P] > [NS/NP]).

Comparisons of the effects of pressure versus GS treatment, with particular attention to potential interaction between these stimuli, were then made. The analysis showed that paired differences between pressurized and unpressurized bones were eliminated in animals infused with GS due to increased resorption in the unpressurized, sham-operated sides ([GS/P] = [GS/NP]). Treatment with GS did not, however, increase the osteoclast profile, and thus differences in osteoclast profile between pressurized and unpressurized bones were not eliminated with GS infusion. Further, no differences were found in the resorption parameters of pressurized bullae treated with GS when compared to pressurized bullae treated with saline ([GS/P] = [NS/P]). The results indicate that pressure and GS are capable of inducing near-identical increases in the osteoclast number and osteoclast surface of bone without interaction or summation. In contrast, GS alone had no effect on osteoclast profile, while pressure (with or without GS) significantly increased the osteoclast profile of bone over controls (Fig 2).

The finding that pressure induces middle ear bone resorption is well supported by previous investigations. For instance, low levels of air pressure were shown by Chole et al,[18] Chole and Chan,[19] and Wolfman and Chole[20] to stimulate bone resorption in the middle ear bullae of Mongolian gerbils. Pressure has also been shown to induce localized bone resorption in the molar root sockets of adult rats.[21] While the underlying control mechanisms of such stress-induced cranial bone resorption are not completely understood, the process can be blocked in both gerbilline bulla bone and rat mandibular bone via cyclooxygenase inhibition,[34-36] and in gerbilline bulla bone by interleukin-1 receptor antagonism[37] and nitric oxide synthase blockade (unpublished results).

In Vitro Effects of Guanethidine Sulfate. Guanethidine sulfate was shown by this investigation to have no inductive effects on calcium release from neonate mouse hemicalvaria following 72 hours of incubation. Similarly, no inhibitory effect by GS on PTH-stimulated calcium release was seen at 10^{-8} or 10^{-6} mol/L (Table 1). The findings suggest that the agent exerts no direct stimulatory activity on murine hemicalvarial bone resorption. Most important, because the agent has been shown to induce bone resorption in vivo, the lack of any direct stimulatory activity on calcium release supports the investigation's thesis of sympathectomy-induced membranous bone resorption in the gerbilline middle ear.

CONCLUSIONS

This investigation has shown the sympatholytic agent GS to have no in vitro activity using the mouse hemicalvarial calcium release assay. With the gerbilline auditory bulla as an in vivo model, GS treatment was shown to significantly increase the osteoclast number and osteoclast surface, but not the osteoclast profile, of middle ear bone without interaction or summation with resorption induction by pressure. The principal hypothesis, that sympathetic fibers innervating bone and periosteum are potential modulators of bone resorption, is therefore supported. The results are in agreement with the findings of our previous investigation, which showed surgical sympathectomy to similarly increase the osteoclast number and osteoclast surface of bone without an effect on the osteoclast profile.[31]

REFERENCES

1. Chole RA. Osteoclasts in chronic otitis media, cholesteatoma, and otosclerosis. Ann Otol Rhinol Laryngol 1988;97:661-6.

2. Klein DC, Raisz LG. Prostaglandins: stimulation of bone resorption in tissue culture. Endocrinology 1970;88:1436-40.

3. Dietrich JW, Goodson JM, Raisz LG. Stimulation of bone resorption by various prostaglandins in organ culture. Prostaglandins 1975;10:231-40.

4. Raisz LG, Dietrich JW, Simmons HA, Seyberth HW, Hubbard W, Oates JA. Effect of prostaglandin endoperoxides and metabolites on bone resorption in vitro. Nature 1977;267:532-4.

5. Meghji S, Sandy JR, Scutt AM, Harvey W, Harris M. Stimulation of bone resorption by lipoxygenase metabolites of arachidonic acid. Prostaglandins 1988;36:139-49.

6. Ren W, Dziak R. Effects of leukotrienes on osteoblastic cell proliferation. Calcif Tissue Int 1991;49:197-201.

7. Gowen M, Mundy GR. Actions of recombinant interleukin 1, interleukin 2, and interferon gamma on bone resorption in vitro. J Immunol 1986;136:2478-82.

8. Gowen M, Mundy GR. Control of bone resorption by recombinant immune cell products: interleukin 1, interleukin 2, and interferon gamma. Bone 1986;7:157.

9. Konig A, Muhlbauer RC, Fleisch H. Tumor necrosis factor alpha and interleukin-1 stimulate bone resorption in vivo as measured by urinary [^{3}H] tetracycline excretion from prelabeled mice. J Bone Miner Res 1988;3:621-7.

10. Fried RM, Voelkel EF, Rice RH, Levine L, Gaffney EV, Tashjian AH. Two squamous cell carcinomas not associated with humoral hypercalcemia produce potent bone resorption–stimulating factor which is interleukin-1a. Endocrinology 1989;125:742-51.

11. Pfeilschifter J, Chenu C, Bird A, Mundy GR, Roodman GD. Interleukin-1 and tumor necrosis factor stimulate the formation of human osteoclast-like cells in vitro. J Bone Miner Res 1989;4:113-8.

12. Tatakis DN, Schneeberger G, Dziak R. Recombinant interleukin-1 stimulates prostaglandin E2 production by osteoblastic cells: synergy with parathyroid hormone. Calcif Tissue Int 1988;42:358-62.

13. Tashjian AH, Voelkel EF, Lazzaro M, Goad D, Bosma T, Levine L. Tumor necrosis factor alpha (cachectin) stimulates bone resorption in mouse calvaria via a prostaglandin-mediated mechanism. Endocrinology 1987;120:2029-39.

14. Shen V, Kohler G, Jeffrey JJ, Peck WA. Bone-resorbing agents promote and interferon gamma inhibits bone cell collagenase production. J Bone Miner Res 1988;3:657-66.

15. Tashjian AH, Voelkel EF, Lazzaro M, et al. Alpha and beta human transforming growth factors stimulate prostaglandin production and bone resorption in cultured mouse calvaria. Proc Natl Acad Sci U S A 1982;13:4535-8.

16. Pfeilschifter J, Mundy GR. Modulation of type 13–transforming growth factor activity in bone cultures by osteotropic hormones. Proc Natl Acad Sci U S A 1987;84:2024-8.

17. Chenu C, Pfeilschifter J, Mundy GR, Roodman GD. Transforming growth factor beta inhibits formation of osteoclast-like cells in long term human marrow culture. Proc Natl Acad Sci U S A 1988;85:5683-7.

18. Chole RA, McGinn MD, Tinling SP. Pressure-induced bone resorption in the middle ear. Ann Otol Rhinol Laryngol 1985;94:165-70.

19. Chole RA, Chan DE. Rapid induction of localized bone resorption in the auditory bulla of the mongolian gerbil, *Meriones unguiculatus*, by increased air pressure. Calcif Tissue Int 1989;45:318-23.

20. Wolfman DE, Chole RA. Osteoclast stimulation by positive middle-ear air pressure. Arch Otolaryngol Head Neck Surg 1986;112:1037-42.

21. Kvam E. Cellular dynamics on the pressure side of the rat periodontium following experimental tooth movement. Scand J Dent Res 1972;80:369-83.

22. Johnson EM, O'Brien F, Werbitt R. Modification and characterization of the permanent sympathectomy produced by the administration of guanethidine to newborn rats. Eur J Pharmacol 1976;37:45-54.

23. Burnstock G, Evans B, Gannon BJ, Heath JW, James V. A new method of destroying adrenergic nerves in adult animals using guanethidine. Br J Pharmacol 1971;43:295-301.

24. Evans BK, Heath JW, Burnstock G. Re-innervation following guanethidine-induced sympathectomy of adult rats. J Neurocytol 1979;8:381-400.

25. Manning PT, Powers CW, Schmidt RE, Johnson EM. Guanethidine-induced destruction of peripheral sympathetic neurons occurs by an immune-mediated mechanism. J Neurosci 1983;3:714-24.

26. Schmidt RE, Summerfield AL, Hickey WF. Ultrastructural and immunohistologic characterization of guanethidine-induced destruction of peripheral sympathetic neurons. J Neuropathol Exp Neurol 1990;49:150-67.

27. Singh IJ, Klein RM, Herskovits MS. Autoradiographic assessment of ^{3}H-proline uptake by osteoblasts following guanethidine-induced sympathectomy in the rat. Cell Tissue Res 1981;216:215-20.

28. Singh IJ, Herskovits MS, Chiego DJ, Klein RM. Modulation of osteoblastic activity by sensory and autonomic innervation of bone. Prog Clin Biol Res 1982;101:535-51.

29. Herskovits MS, Singh IJ. Effect of guanethidine-induced sympathectomy on osteoblastic activity in the rat femur evaluated by ^{3}H-proline auto-radiography. Acta Anat (Basel) 1984; 120:151-5.

30. Hill EL, Turner R, Elde R. Effects of neonatal sympathectomy and capsaicin treatment on bone remodeling in rats. Neuroscience 1991;44:747-55.

31. Sherman BE, Chole RA. In vivo effects of surgical sympathectomy on intramembranous bone resorption. Am J Otol 1996;17:343-6.

32. Parfitt AM. Bone histomorphometry: proposed system for standardization of nomenclature, symbols, and units. Calcif Tissue Int 1988;42:284-6.

33. Johnson EM, Manning PT. Guanethidine-induced destruction of sympathetic neurons. Int Rev Neurobiol 1984;25:1-37.

34. Adachi K, Chole RA, Yee J. Indomethacin inhibition of middle ear bone resorption. Arch Otolaryngol Head Neck Surg 1991;17:267-9.

35. Jungkeit MC, Chole RA. Ibuprofen inhibits localized bone resorption in the middle ear. Calcif Tissue Int 1991;48: 267-71.

36. Yamasaki K, Miura F, Suda T. Prostaglandin as a mediator of bone resorption induced by experimental tooth movement in rats. J Dent Res 1980;59:1635-42.

37. Chole RA, Faddis BT, Tinling SP. In vivo inhibition of localized bone resorption by human recombinant interleukin-1 receptor antagonist. J Bone Miner Res 1997;10:281-4.

Ann Otol Rhinol Laryngol 108:1999

LOCAL CORTICOSTEROID TREATMENT OF CAUSTIC INJURIES OF THE ESOPHAGUS

A PRELIMINARY REPORT

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So far, no therapy has been shown to reduce the incidence of strictures in the esophagus after ingestion of caustic agents. Two patients with pronounced caustic ingestion injuries were treated locally with solutions of corticosteroids normally used for inhalation therapy in lung diseases. Serious strictures did not appear, and their swallowing returned to normal. Further studies are needed.

KEY WORDS — caustic esophagitis, caustic ingestion, corticosteroids, local treatment.

INTRODUCTION

The final result of severe caustic injuries of the esophagus will often be serious strictures or complete occlusion of the esophagus.[1]

There are many controversies in the treatment of these injuries: time of endoscopy, feeding of the patient, administration of antibiotics and steroids, and the use of nasogastric (NG) tubes.[2-6]

As far as I know, there are no reports on early local corticosteroid treatment after caustic ingestion injuries. I therefore wish to present 2 cases in which the patients were given solutions of corticosteroids with high anti-inflammatory potency — beclomethasone dipropionate (BDP) and budesonide, normally used for inhalation therapy in obstructive lung diseases — as a local therapy in the esophagus after caustic ingestion injuries.

CASE REPORTS

Case 1. A 66-year-old woman, in a suicide attempt, tried to dissolve pearls of sodium hydroxide in hot water and then drank the solution. She was brought to a local hospital and was intubated because of inspiratory stridor and laryngeal edema. After admission to our hospital, endoscopy was performed a few hours later. Caustic injuries were seen in the mouth and pharynx with mucosal edema, ulcerations, and hemorrhage. There was heavy edema of the larynx and at the entrance of the esophagus. The esophageal burns were circular and deep, with the swollen mucosa partly detached and ulcerated with hemorrhage. The esophagus was cleared of undissolved pearls. A soft NG tube was inserted, fluids and antibiotics (cefuroxime sodium 750 mg 3 times per day) were given intravenously, and a tracheotomy was performed. She was carefully examined for the development of gastric perforation, mediastinitis, and other complications. A liquid diet was given through the tube and omeprazole (20 mg/d) was given to avoid gastric reflux. As soon as she was able to (after 8 days), she was allowed to drink bypassing the tube. An antibiotic (phenoxymethyl penicillin solution; 1 g twice per day) and an antimycotic (nystatin; 100,000 IU 4 times per day) were given orally for 1 month. A solution of BDP (50 µg/mL) was given orally (250 µg twice per day) for 2 months. The intention was to let this solution stay in the esophagus as long as possible, so it was given at the end of meals.

Endoscopy was performed regularly, the first after 3 weeks, to examine stricture formation. The tip of the epiglottis had a necrotic surface and there was moderate edema in the larynx. The upper part of the esophagus had a slight necrotic surface, but no strictures were seen. The patient was decannulated and talked with a whispering voice. The NG tube was left in place for 1 month.

At repeated endoscopies there was a tendency toward 2 short strictures: 1 at the entrance and 1 in the upper part of the esophagus. They were dilated quite easily. A last endoscopy was performed after 4 months, with no signs of strictures. Her depression was treated and her voice gradually returned to normal. At follow-ups after 2 and 5 years, she had no swallowing complaints.

Case 2. A 64-year-old woman, by accident, drank a strong stable detergent with a pH greater than 14. She tried to spit out the solution and noted burns on her lips and in her mouth. Over the next 2 weeks, she noted gradually increasing problems with swallowing solid food. She vomited after ingestion and lost 3 kg of weight. An x-ray demonstrated a pronounced stenosis of the esophagus with a proximal

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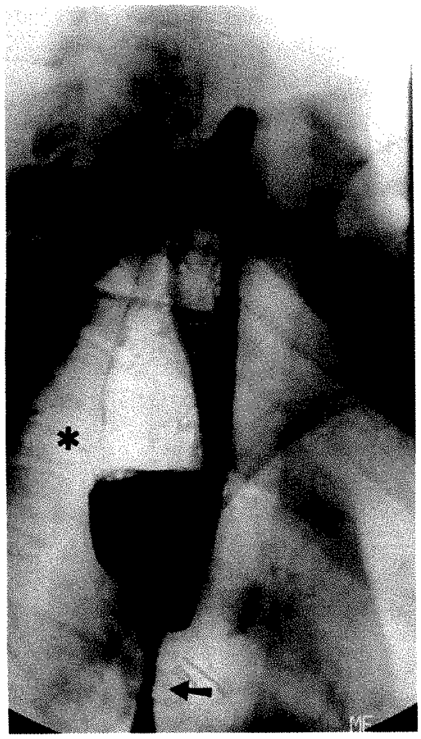

Fig 1. (Case 2) Esophagram 2 weeks after ingestion of strong alkaline solution, demonstrating pronounced stenosis (arrow) with proximal dilatation (asterisk) and reduced distal motility.

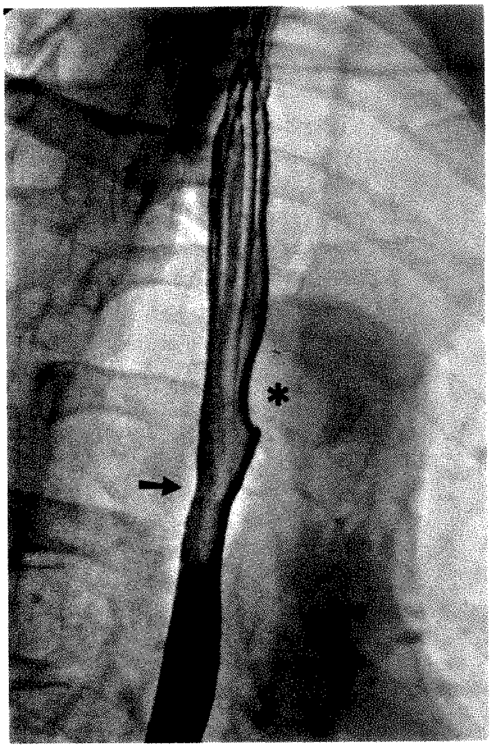

Fig 2. (Case 2) Follow-up esophagram after 14 months, showing slight impression in wall (arrow) of esophagus, but no stricture. Asterisk — impression by aorta.

dilatation and reduced distal motility (Fig 1). Endoscopic examination revealed edematous changes of the mucosa, with evidence of necrosis and almost complete occlusion of the esophagus within a limited region, roughly 25 cm below the teeth. Dilation was performed and a soft NG tube was inserted. She was allowed to drink, bypassing the tube. Ranitidine hydrochloride (150 mg twice per day) was given through the tube to avoid gastric reflux.

An antibiotic (cefuroxime sodium 1.5 g 3 times per day) was given intravenously for 10 days. Budesonide in solution (0.5 mg/mL) was given orally (2.5 mg twice per day), bypassing the tube. A new endoscopy was performed after 3 weeks. The mucosa of the esophagus was now normal and showed no edema. A short stricture was dilated quite easily, and afterward, the patient had no problem eating. The NG tube was left in place for 7 weeks, and budesonide was given over this time period. After 2 months, a new endoscopy with dilation was performed without complications. A last follow-up was done after 14 months. A new esophagram showed a slight impression in the wall of the esophagus, but no stricture (Fig 2). The patient had no swallowing problems and had gained weight.

DISCUSSION

It is a very difficult therapeutic dilemma to determine the most appropriate management of caustic ingestion injuries. The literature shows many conflicting opinions, but no therapy has been shown, so far, to reduce the incidence of strictures after ingestion of caustic agents.[2]

It is generally accepted that endoscopy is the most reliable and accurate method to determine the extent of esophagal injury, but the timing of endoscopy is controversial. Friedman[3] suggests endoscopy be performed between 24 and 48 hours following ingestion, to provide the most information and to ensure the highest degree of patient safety. This is because an earlier examination may lead to an underestimation of the injury, and delayed examination may increase the risk of iatrogenic injury. Other authors[4-6] suggest an early endoscopy, within 24 hours.

There is also a controversy about the feeding of the patient. Some authors[4,5] prescribe no food by mouth and administration of intravenous fluids. The esophagus may be permitted to rest for several days after a severe injury in order to avoid contamination of the wound and damage to the regenerating epithelium. Wijburg et al[6] put their patients on a liquid diet. They also inserted a specially made silicone rubber NG tube as a stent when esophageal burns appeared to be circular and deep.

The third controversy is the administration of antibiotics. Friedman[3] recommends withholding antibiotics until the patient develops signs or symptoms of secondary infection. Sugawa and Lucas,[4] on the other hand, treat their patients with intravenous antibiotics immediately. The administration of systemic corticosteroids remains controversial. Some studies conclude that steroids are not useful for the prevention of esophageal strictures,[2,6] but Friedman[3] admits, with hesitation, that steroids may be useful in transmucosal injuries. Ferguson et al[5] concluded that neither antibiotics nor steroids appear to alter the clinical course in these patients. Ochi et al[1] treated 1 patient with extensive damage with both antibiotics and steroids intravenously, without any visible effect.

There are indications in studies on pigs that immediate local corticosteroid treatment may reduce the effect in the lung of chemical toxic injury induced by chlorine gas.[7] Corticosteroids can affect every stage of inflammatory reactivity; they influence the movement and distribution of inflammatory cells, and they also diminish the production of inflammatory mediators and reduce their effects. Steroids also inhibit the leakage of fluid and cells from capillary beds.[8] The steroids I used have high local potency (affinity to glucocorticoid receptors and activity in vasoconstrictor tests). After topical application (skin blanching test), BDP is about 600 times more potent, and budesonide more than 1,000 times more potent, than hydrocortisone in inducing vasoconstriction.[9] This would suggest a high potency for anti-inflammatory effects on the esophageal mucosa and submucosa.

In the first patient, I performed an endoscopy as soon as possible and was able to clear the esophagus of solid lye. I therefore consider an early endoscopy to be of great value. A liquid diet was tried, bypassing a soft NG tube to encourage normal swallowing. Antibiotics were used both intravenously (both cases) and orally (case 1). The prolonged treatment with oral antibiotics and antimycotics may, in retrospect, seem to be overtreatment. I tried local treatment in both cases with corticosteroids with high anti-inflammatory properties on the basis of experience obtained from animal experiments.

At repeated endoscopies in both patients, there was a considerable regression of the mucosal damage. The patients did not develop persisting strictures, a common complication after caustic ingestion injuries, although I considered the initial damage to be extensive and deep. This might be an overestimation, although in the first case the suicidal adult purposefully ingested a large volume of sodium hydroxide. In both patients, swallowing returned to normal.

I have seen no disadvantages with the local corticosteroid treatment, but further studies are needed and will be carried out.

REFERENCES

1. Ochi K, Ohashi T, Sato S, Watarai J, Maeda C, Takeyama I. Surgical treatment for caustic ingestion injury of the pharynx, larynx, and esophagus. Acta Otolaryngol Suppl (Stockh) 1996 (suppl 522):116-9.

2. Kikendall JW. Caustic ingestion injuries. Gastroenterol Clin North Am 1991;20:847-57.

3. Friedman EM. Caustic ingestion and foreign bodies in the aerodigestive tract of children. Pediatr Clin North Am 1989; 36:1403-10.

4. Sugawa C, Lucas CE. Caustic injury of the upper gastrointestinal tract in adults: a clinical and endoscopic study. Surgery 1989;106:802-7.

5. Ferguson MK, Migliore M, Staszak VM, Little AG. Early evaluation and therapy for caustic esophageal injury. Am J Surg 1989;157:116-20.

6. Wijburg FA, Heymans HSA, Urbanus NAM. Caustic esophageal lesions in childhood: prevention of stricture formation. J Pediatr Surg 1989;24:171-3.

7. Gunnarsson M, Walther S, Holmström A, Jansson I, Lennquist S. Nebulized corticosteroid improves pulmonary function after chlorine gas exposure in pigs [Abstract]. Prehospital Disaster Med 1993;8:95-6.

8. Svedmyr N. Effects of glucocorticoids on the airways. Eur J Respir Dis 1982;63(suppl 122):48-53.

9. Johansson SÅ, Andersson KE, Brattsand R, Gruvstad E, Hedner P. Topical and systemic glucocorticoid potencies of budesonide, beclomethasone dipropionate and prednisolone in man. Eur J Respir Dis 1982;63(suppl 122):74-82.

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IMAGING CASE STUDY OF THE MONTH

DEVELOPMENTAL CHANGES IN THE ANTRUM OF A CHILD WITH CHRONIC MAXILLARY ATELECTASIS

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INTRODUCTION

Chronic maxillary atelectasis (CMA) is characterized by a persistent decrease in maxillary sinus volume due to inward bowing of one or more antral walls.[1] It is an uncommon condition thought to develop when the medial infundibular wall (MIW) causes valvular occlusion of the natural ostium.[2] We recently reported on the first confirmed case of pediatric CMA and were able to demonstrate that the same rhinoscopic and radiographic features found in adults were also diagnostic of CMA in a child.[3] We documented the presence of negative antral pressure and, as in adult CMA, found evidence of valvular occlusion of the natural ostium by a laterally malpositioned and flimsy MIW.[3]

Complete ostial occlusion by the MIW can be determined by rhinoscopy, computed tomography (CT), or antral manometry, and its presence distinguishes CMA from maxillary sinus hypoplasia (MSH).[3] A review of the recent literature suggests that pediatric CMA may frequently occur in association with MSH.[4,5] Since chronic ostial obstruction is thought to arrest or retard antral pneumatization, CMA that involves a developing antrum would be expected to result in MSH.[6] Whether normal pneumatization resumes after ventilation is restored to a hypoplastic antrum is not known and has been a subject of interest for many decades.[4,6] In this report, we address this issue by radiographically measuring developmental changes in the maxillary sinuses of a child with unilateral CMA and MSH, before and after middle meatal antrostomy.

CLINICOPATHOLOGICAL FINDINGS

A 6-year-old girl was referred by her pediatrician 1 month after she was struck on the nose by a door. She was asymptomatic, and the physical examination findings were unremarkable. A review of the head CT scan done at the time of the injury showed an incidental finding of a small right maxillary sinus with opacification, but no evidence of a nasal fracture. One year later, she was brought in by her parents for recurrent episodes of mild epistaxis. Anterior rhinoscopy revealed a widened right middle meatus due to a lateralized MIW and a retracted fontanel, but no obvious source of bleeding. The family was reassured and advised to return if bleeding recurred or if symptoms of recurrent sinus infection developed. She returned 2 years later complaining of frequent periorbital facial pain and rhinorrhea. Anterior rhinoscopy again showed fontanel retraction on the right. A limited coronal CT scan demonstrated complete right antral opacification, as well as widening of the right middle meatus. The patient received a diagnosis of CMA involving a hypoplastic right antrum based on those findings.

Initial treatment with an antibiotic and a decongestant was unsuccessful, and an endoscopic right middle meatal antrostomy was performed. Intraoperative findings included complete ostial occlusion of the right maxillary sinus by a laterally malpositioned and flaccid-appearing MIW. Marked retraction of the fontanel could easily be seen, and a transient antral opening pressure of –4 cm H_2O was detected by needle manometry.[2] The MIW had prominent height, and the uncinate process lacked an attachment to the inferior turbinate posteriorly. The MIW had to be peeled off the lateral wall with an ostium seeker in order to see the underlying natural ostium. A large amount of thick, gray, tenacious fluid was evacuated from the antrum, and a generous antrostomy was created. The histopathology showed slight chronic inflammatory changes in the mucosa.

She did well postoperatively; however, 1 year after surgery, she developed chronic nasal congestion and rhinorrhea. These symptoms followed an upper respiratory tract infection and persisted despite medical management. A limited CT scan of the paranasal

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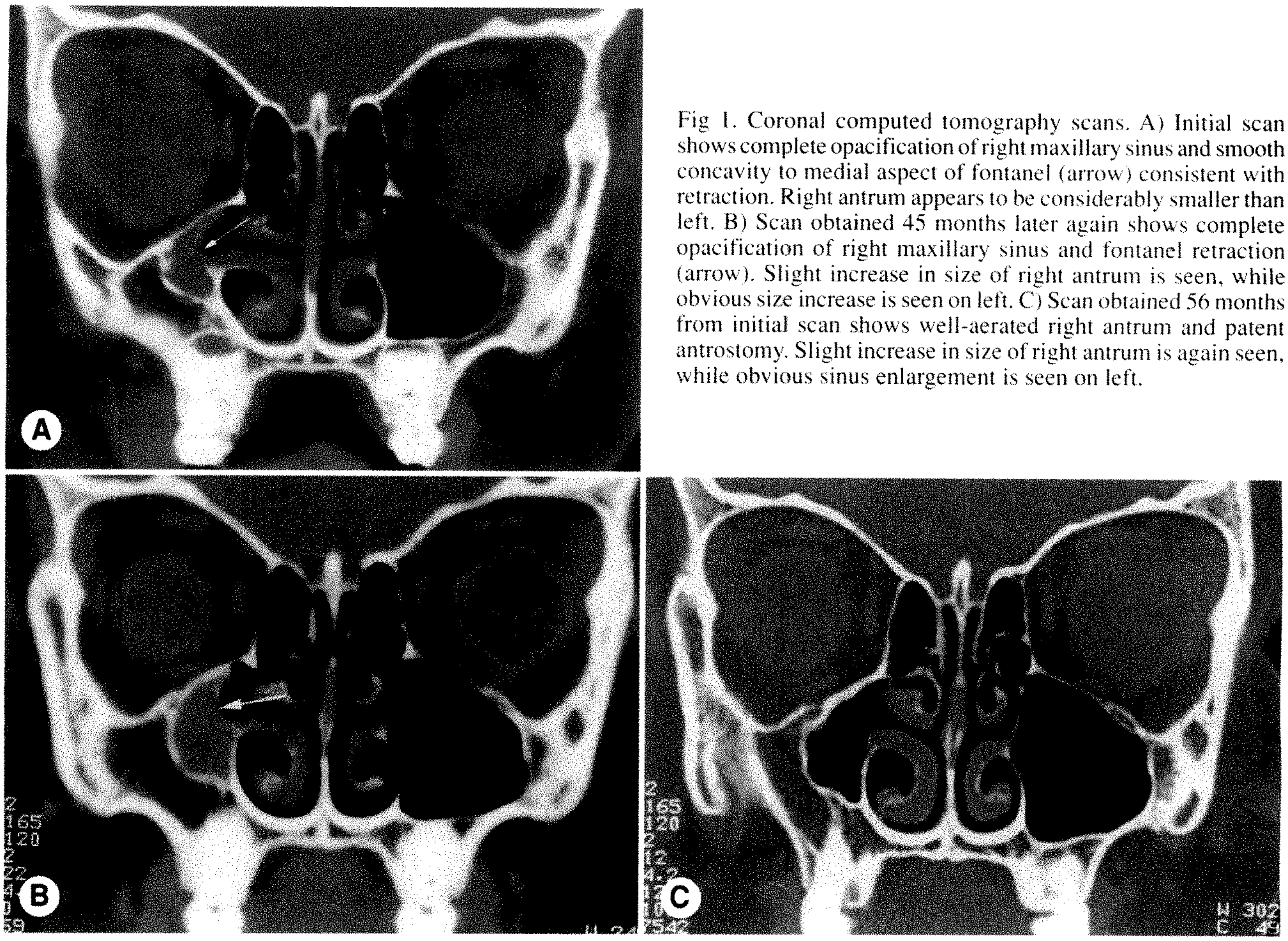

Fig 1. Coronal computed tomography scans. A) Initial scan shows complete opacification of right maxillary sinus and smooth concavity to medial aspect of fontanel (arrow) consistent with retraction. Right antrum appears to be considerably smaller than left. B) Scan obtained 45 months later again shows complete opacification of right maxillary sinus and fontanel retraction (arrow). Slight increase in size of right antrum is seen, while obvious size increase is seen on left. C) Scan obtained 56 months from initial scan shows well-aerated right antrum and patent antrostomy. Slight increase in size of right antrum is again seen, while obvious sinus enlargement is seen on left.

sinuses was done and showed the right antrum to be well aerated, with a widely patent antrostomy. The scan was otherwise unremarkable. She eventually improved following a change in oral antibiotic and decongestant therapy.

RADIOLOGIC FINDINGS

The initial coronal CT scan of the paranasal sinuses showed complete opacification of the right antrum. The right MIW was laterally malpositioned, and the fontanel was markedly retracted. The antrum was smaller in size than the left, and there was more intramedullary bone surrounding it, consistent with incomplete pneumatization (Fig 1A). A coronal CT scan done 45 months later showed minimal change in the appearance of the right antrum, while obvious enlargement was seen in the left maxillary sinus (Fig 1B). A third CT scan performed approximately 1 year after an endoscopic middle meatal antrostomy showed continued enlargement of the left antrum and a slight increase in size of the right (Fig 1C).

DIMENSIONAL MEASUREMENTS OBTAINED AT 3 TIME POINTS OVER 56-MONTH PERIOD

Age	*Maxillary Sinus Volume (mL)*		*Maxillary Sinus Surface Area (cm^2)*	
(y)	*Right*	*Left*	*Right*	*Left*
5.8	1.43 ± 0.02	8.24 ± 0.04	7.97 ± 0.16	19.89 ± 0.02
9.6	2.65 ± 0.02	12.06 ± 0.16	11.67 ± 0.02	26.75 ± 0.25
10.5	2.75 ± 0.01	13.12 ± 0.18	12.84 ± 0.21	28.47 ± 0.07

Data are mean ± SD.

Antral volume and surface area measurements were taken from coronal CT sections (see Table and Fig 2). The scans were traced, and registration (reference) points were digitized manually with an electronic data tablet. Standard computerized planimetry equations were used to determine the area of each region in each section. The volumes were then calculated as the sum of the 2- and 5-mm–thick sections with areas equal to the region areas. This "stacked coin" volume approximation is accurate within 10% of the actual volume. Data were compiled and linear regression lines were plotted with Microsoft Excel software.

The normal-appearing left sinus had a volume of 8.24 mL that increased at an average rate of about 1 mL/y to 13.12 mL over a 56-month period. The volume of the atelectactic right antrum was 1.43 mL (17% of contralateral sinus volume) and increased at an average rate of about 0.3 mL/y to 2.75 mL over the same time interval. The rate of increase in the

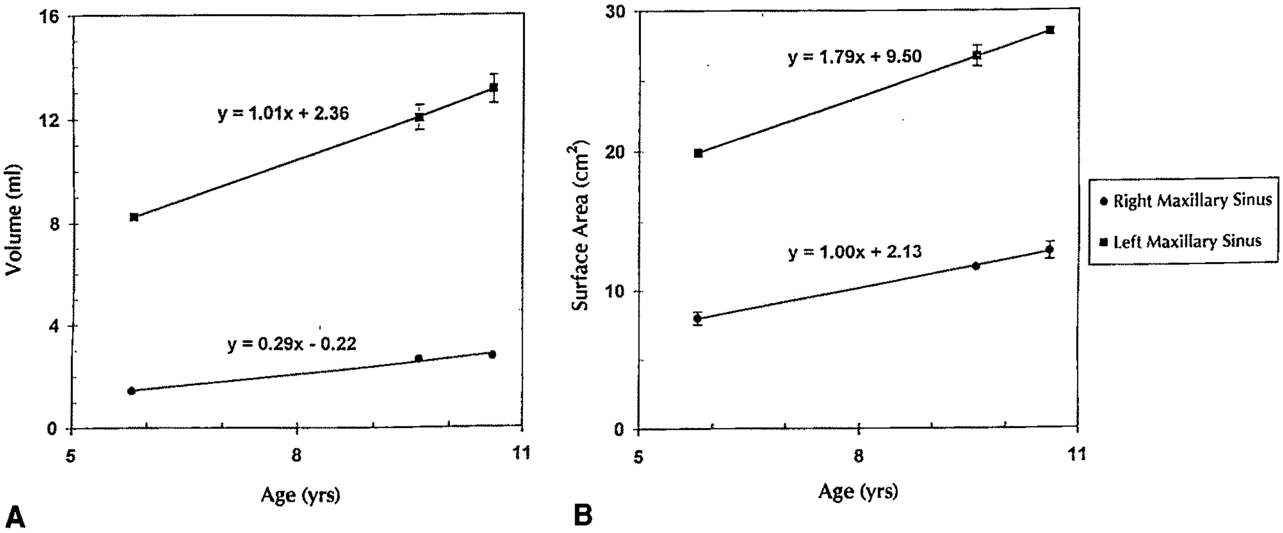

Fig 2. Graphs of maxillary sinus A) volume and B) surface area as function of age.

right antral volume did not change following restoration of sinus ventilation.

DISCUSSION

Prior to our report on the first confirmed case of pediatric CMA, it was not known whether a childhood form of the disorder existed.[3] In the past, fontanel retraction and complete opacification were simply thought to be among a group of radiographic findings that characterized MSH.[7,8] Although an association between CMA and MSH is known to exist,[1,3] these entities can occur independently.[2,7] A causal relationship is strongly suspected, but remains to be proven.

The cause of CMA is not known, but a deficiency in the posterior attachment of the uncinate process to the inferior turbinate bone is considered to be a key predisposing factor.[2,3] This deficiency may be congenital in children with CMA.[1,3] Prominent height of the posterior aspect of the infundibular wall along with a narrow infundibular passage are also considered to be important contributors.[2,3] Middle meatal inflammation is postulated to incite flap-valve obstruction of the natural ostium and set the stage for a self-perpetuating occlusive process not unlike that of chronic otitis media with effusion due to eustachian tube dysfunction.[1-3]

Diagnostic features of CMA[1] were radiographically demonstrated in this case presentation. They included a laterally deviated MIW, fontanel retraction, and antral opacification. The right maxillary sinus was also hypoplastic, and our growing clinical experience indicates that antral underdevelopment may be found in virtually all pediatric patients with CMA[9] (also Kass et al, unpublished observations).

This case addresses the question of whether resumption of antral ventilation affects the rate of pneumatization in a hypoplastic maxillary sinus. In the 45-month interval prior to the middle meatal antrostomy, the volumetric growth rate on the right was less than one third the rate on the left. In the 11-month period following endoscopic sinus surgery, essentially no change in volumetric growth rate occurred. This would suggest that the ventilatory status of the antrum does not influence pneumatization and that hypoplasia results from some intrinsic process. An alternative, and perhaps more plausible, explanation of the findings is that complete ostial occlusion for any significant period of time during development irreversibly hampers antral bone remodeling and thereby diminishes the volumetric growth rate of the sinus. Further study with more patients and greater long-term follow-up is needed before any firm conclusions can be drawn.

At this stage in our understanding, we believe the decision to treat pediatric CMA should be based on the presence of associated symptoms and recurrent sinus infections. Restoring ventilation solely for the purpose of trying to maximize antral development does not appear to be warranted, for 3 reasons. First, the case presented suggests that restoration of ventilation to an already hypoplastic antrum may have little or no impact on growth rate. Second, MSH per se is not associated with clinically significant sequelae. Last, there is no evidence to indicate that untreated pediatric CMA will lead to orbital or cosmetic deformities, as can occur in the adult form of the disorder.

Treatment goals for children with CMA are the same as for adults, namely, to relieve symptoms and prevent recurrent infections by achieving complete antral drainage and good ventilation. Since valvular occlusion is theoretically reversible, medical treatment with antibiotic and decongestant therapy may be warranted; however, for those patients who fail to respond, the problem can be corrected with an endoscopic middle meatal antrostomy. This is a relatively safe and simple procedure, with minimal associated morbidity, provided that the distorted anatomy is recognized and proper technique is used to avoid inadvertent entry into the orbit.

REFERENCES

1. Kass ES, Salman S, Rubin PAD, Weber AL, Montgomery WW. Chronic maxillary atelectasis. Ann Otol Rhinol Laryngol 1997;106:109-16.
2. Kass ES, Salman S, Montgomery WW. Manometric study of complete ostial occlusion in chronic maxillary atelectasis. Laryngoscope 1996;106:1255-8.
3. Kass ES, Salman S, Montgomery WW. Chronic maxillary atelectasis in a child. Ann Otol Rhinol Laryngol 1998;107:623-5.
4. Proctor T, Naclerio R. Development of a hypoplastic maxillary sinus. Ann Otol Rhinol Laryngol 1996;105:327-8.
5. Milczuk HA, Dalley RW, Wessbacher FW, Richardson MA. Nasal and paranasal sinus anomalies in children with chronic sinusitis. Laryngoscope 1993;103:247-52.
6. Wasson WW. Changes in the nasal accessory sinuses after birth. Arch Otolaryngol 1933;17:199-211.
7. Bolger WE, Woodruff WW, Morehead J, Parsons DS. Maxillary sinus hypoplasia: classification and description of associated uncinate process hypoplasia. Otolaryngol Head Neck Surg 1990;103:759-65.
8. Wake M, Shankar L, Hawke M, Takeno S. Maxillary sinus hypoplasia, embryology, and radiology. Arch Otolaryngol Head Neck Surg 1993;119:1353-7.
9. Kass ES. Diagnosis and treatment of chronic maxillary atelectasis in adults and children. Curr Opin Otolaryngol Head Neck Surg 1999;7:39-43.

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CLINICOPATHOLOGICAL CONSULTATION

REVIEW OF SINUS HISTIOCYTOSIS WITH MASSIVE LYMPHADENOPATHY (ROSAI-DORFMAN DISEASE) OF HEAD AND NECK

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The entity known as sinus histiocytosis with massive lymphadenopathy (SHML), or Rosai-Dorfman disease (RD disease), is an uncommon benign proliferation of hematopoietic and fibrous tissue that often presents in the head and neck region. Its initial manifestations most often include a roughly symmetric, painless, bilateral cervical adenopathy, although extranodal disease may develop in a minority of patients. The key histologic feature of SHML is the presence of various numbers of large, pale histiocytic cells that contain within their cellular borders apparently engulfed lymphocytes ("emperipolesis"); these distinctive large, pale cells — RD cells — are S-100 protein–positive by immunostaining and so differ from ordinary histiocytes. Despite its sometimes impressive clinical presentation, SHML is a benign and self-limited disease, whose treatment is aimed largely at controlling its local manifestations (most often by surgical therapy). The microscopic differential diagnosis, particularly in extranodal disease, is at times challenging and can include Langerhans' cell histiocytosis, Hodgkin's disease, non-Hodgkin's lymphoma, metastatic carcinoma, and metastatic malignant melanoma.

HISTORICAL BACKGROUND AND TERMINOLOGY

Sinus histiocytosis with massive lymphadenopathy (SHML) was established as a distinct clinicopathological entity by Rosai and Dorfman[1,2] in 1969. As a tribute to the clarity of these authors' original descriptions, SHML is also known as Rosai-Dorfman disease (RD disease), and so these two terms are used interchangeably in this article.

Although the cause of SHML remains unknown, the disease is thought to be a disorder of the mononuclear phagocyte and immunoregulatory effector (M-PIRE) system.[3] Indeed, SHML is classified as an idiopathic "histiocytosis" that may be recognized morphologically by its diagnostic large "histiocyte-like" cells, the so-called Rosai-Dorfman (RD) cells.[4] Whereas several findings suggest a relationship of RD cells with the M-PIRE system, the peculiar antigenic profile of RD cells makes it difficult to identify their normal cellular counterpart (professional phagocytic cells versus immune accessory cells) or to define the precise nosologic classification of SHML.[5]

Lymph nodes are frequently affected at presentation. However, the disease may occur at extranodal sites (ESHML) as well, even without any clinical involvement of lymph nodes. In these instances, the absence of lymphadenopathy makes the term SHML inappropriate; thus, for extranodal disease the eponym RD disease is preferable.[6]

EPIDEMIOLOGY

Although the first cases were identified in South Africa,[1] RD disease has a widespread geographic distribution.[6-9] Comprehensive study of patients with RD disease has been made possible through the establishment of a registry that now contains records and pathological samples from over 600 cases.[4,10] More than half of them are from the United States and Western Europe, but there is also a disproportionately high number of cases from Africa and the Caribbean region.[10] An unusual referral pattern might have contributed to the unusually high incidence of this entity in some geographic areas.[4]

Rosai-Dorfman disease affects all age groups, but is more common in children and adolescents than in adults[10,11]; exclusive extranodal disease is more frequent in elderly individuals. The youngest patient had congenital disease, and the oldest developed

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TABLE 1. MORPHOLOGICAL AND PHENOTYPIC DIAGNOSTIC FEATURES OF RD DISEASE

Pathological Investigation	*Morphological Findings*	*Diagnostic Interpretation*
Low magnification	Lymph nodes: sinuses expanded by polymorphous cellular infiltration	Suggestive of RD disease
	Extranodal sites: alternating pale and dark areas	Suggestive of RD disease
High magnification	Typical polygonal RD cells	Single most important finding in diagnosing RD disease
	Emperipolesis	Suggestive of but not very specific to RD disease
Immunostaining	S-100 protein reactivity	Useful additional finding when polygonal RD cells with evident emperipolesis are not easily evidenced

RD — Rosai-Dorfman.

symptoms at age 74.[4] Rosai-Dorfman disease has been reported in siblings[12] and identical twins.[13]

The registry in 1990 contained an equal number of black and white patients. Males are slightly more commonly affected than females.[4]

The association with immune disorders, simultaneous with or preceding clinical onset of RD disease, has been documented in the registry. The most common immune abnormalities are the presence of autoantibodies (against red cell antigens) and joint disease (from mild polyarthralgia to extensive, disabling arthritis). Additional abnormalities include unusually severe infections (most commonly pneumonia), glomerulonephritis, asthma, and juvenile-onset diabetes mellitus.[4,14]

Positive serologic tests for Epstein-Barr virus (EBV) have been documented in some patients with RD disease, especially in those with associated immune disorders.[4] By molecular techniques, in pathologically proved cases of RD disease, no evidence of latent or lytic EBV carriage in the RD cells and/or in the background lymphocytes has been demonstrated. The reported serologic evidence of EBV infection might therefore represent an epiphenomenon of EBV superinfection or perhaps a manifestation of viral reactivation in the presence of an abnormality in the patient's immune control system (which is itself commonly found in such patients).[15]

Human herpesvirus 6 has been detected in RD disease tissues,[16] but this organism is so commonly present in lymphoid tissue that the significance of this finding remains dubious.[10]

PATHOLOGICAL FINDINGS

Gross Findings of Nodal Disease. The gross examination of the removed lymph nodes shows 1 or more enlarged lymph nodes, which often are matted together by a prominent perinodal and capsular fibrosis. The cut surface is nodular or diffusely homogeneous and varies from gray to golden yellow, depending on the amount of fat present.[4,6,10]

Gross Findings of Extranodal Disease. The gross appearance of the lesions varies, and so they may present as nodular, sessile, polypoid, or exophytic growths with a pale gray to yellow appearance. In the skin, the lesion presents as solitary or multiple papules, plaques, or nodules.

Microscopic Findings of Nodal Disease. The lymph node capsule can be thickened and fibrotic. Fibrosis may extend to perinodal soft tissues, but it usually does not extend into the lymph node parenchyma, although areas of fibrotic change may occasionally appear.[4,6,10,11,17]

Lymph node sinuses are expanded by a polymorphous cellular infiltrate composed of neutrophils, lymphocytes, plasma cells, medium-sized mononuclear cells with indented nuclei, and, typically, numerous large and distinctive RD cells (Table 1 and Fig 1).

Rosai-Dorfman cells have round-to-oval, medium-to-large nuclei with a vesicular chromatin pattern. The nuclear membranes are well delineated, due to chromatin margination. Occasionally, RD cells may show binucleation. Most nuclei contain a centrally located, single nucleolus. It is eosinophilic and small, but distinct and sometimes prominent. Nuclear atypia is unusual. Cytoplasm is abundant, and with hematoxylin and eosin staining, it appears lightly eosinophilic or, less frequently, amphophilic or clear. Many RD cells have granular-appearing cytoplasm and irregular, poorly defined cell borders. Variable numbers of cells with vacuolated foamy cytoplasm, due to a high content of neutral lipid, may be noticed.[4,6,10,11,17]

Phagocytized intact-appearing lymphocytes are present in the cytoplasm of most RD cells (lymphocytophagocytosis or emperipolesis: "wandering about within"; Table 1 and Fig 1B). Internalized lymphocytes are usually located within cytoplasmic vacuoles.[18] Less often, cytoplasm may contain plasma cells, neutrophils, and red blood cells (cytophagocytosis).[6] Although not pathognomonic (and more

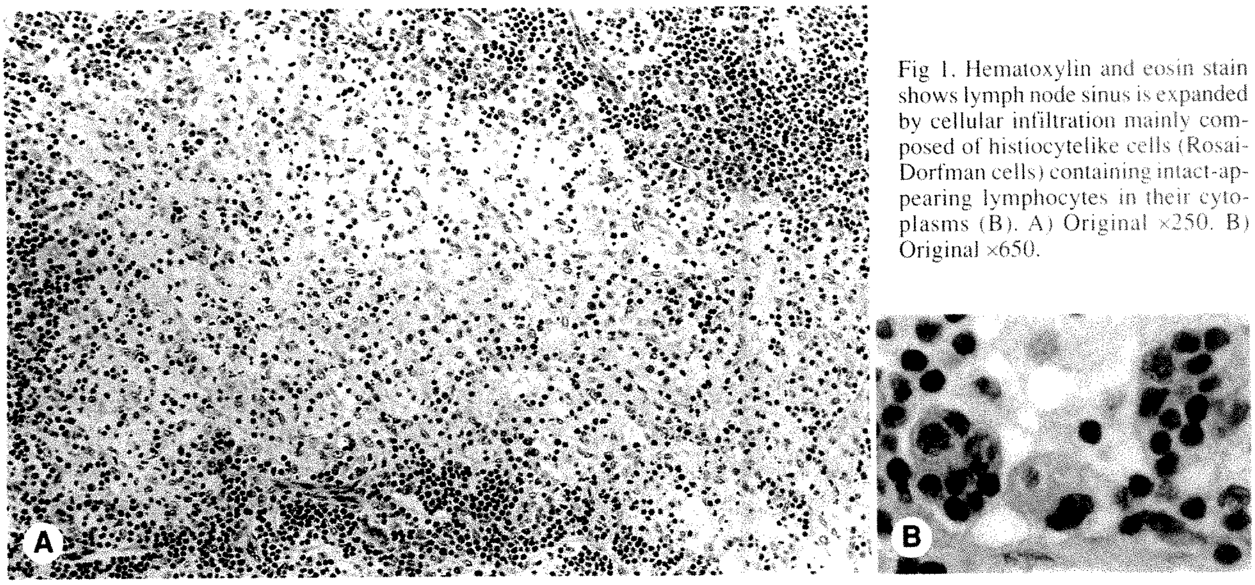

Fig 1. Hematoxylin and eosin stain shows lymph node sinus is expanded by cellular infiltration mainly composed of histiocytelike cells (Rosai-Dorfman cells) containing intact-appearing lymphocytes in their cytoplasms (B). A) Original ×250. B) Original ×650.

easily appreciated in S-100 protein immunohistochemical stains), this feature is of great diagnostic importance (J. K. C. Chan, personal communication, 1998).

In the early stages, the lymph node architecture is usually preserved, whereas in RD disease of long duration, proliferation of RD cells may massively dilate the sinuses, with complete effacement of normal nodal architecture.[6]

The intersinusal lymphoid tissue contains a variable, sometimes impressive, number of plasma cells that tend to aggregate around high endothelial venules. Lymphoid follicules are usually lacking, but a few residual germinal centers may be noticed. Whereas eosinophils are virtually absent, focal aggregates of neutrophils may be present, occasionally forming microabscesses.[6]

Microscopic Findings of Extranodal Disease. The lesions are often sharply circumscribed or demarcated, although sometimes they may have ill-defined margins.[19]

In whatever extranodal site, RD disease can be suspected in most cases at low magnification when there is a characteristic pattern of alternating pale and dark areas. Whenever this pattern is seen, diagnostic RD cells must be looked for at high magnification.[20] The morphological features are reminiscent of those of affected lymph nodes in that they superficially resemble the appearance of lymphoid tissue affected by RD disease (albeit appearing in an extranodal site); on close scrutiny, however, these extranodal lesions of RD disease lack the architecture of true nodal tissue and so may be distinguished from disease appearing in an actual lymph node.[4] In extranodal RD, there are often dilated spaces similar to the dilated channels of actual lymph nodes, filled with many characteristic RD cells (pale-staining areas) alternating with lymphoid tissue rich in plasma cells (dark-staining areas) with scattered reactive germinal centers.[6,11] However, fibrosis is usually greater in extranodal sites, and the number and prominence of the diagnostic cells tend to be less than in nodal involvement.[4,6,19,21]

Lymphocyte phagocytosis is identified in all cases, but varies from prominent and readily identifiable to less conspicuous and difficult to recognize, owing to difficulties in defining cell borders.[21]

The process is devoid of significant cellular pleomorphism, necrosis is absent, and mitotic activity is inconspicuous.[19,21] However, RD cells can exhibit some degree of nuclear atypia; this is not to be misinterpreted as a feature of malignancy.[20]

Older lesions of extranodal RD disease can be difficult to diagnose because of the paucity of diagnostic RD cells, the presence of foamy histiocytes, and secondary changes, including fibrosis, causing spindling of cells.[20]

If nodal RD disease is already diagnosed, typical RD cells in a characteristic milieu, though rare, are diagnostic for tissue involvement by RD disease. Conversely, in the absence of a diagnostic lymph node, larger numbers of characteristic RD cells, with clear evidence of cytophagocytosis, are required (Tables 1 and 2).[4]

Cultures[4,22] and histochemical stains for bacterial, mycobacterial, and fungal microorganisms, including Gram, periodic acid–Schiff with and without diastase, Gomori's methenamine silver, Grocott, Ziehl-Neelsen, and Warthin-Starry, are consistently nega-

TABLE 2. MISLEADING FEATURES IN EXTRANODAL RD DISEASE

Pathological Findings	*Misleading Features*	*Comments*
Extensive fibrosis	Histiocyte-like cell spindling	Look for intermingled polygonal RD cells
Poorly defined cell borders	Lymphophagocytosis not prominent	Apply immunohistochemistry to enhance RD cell borders
Nuclear atypia	Possible misdiagnosis as neoplastic proliferation	Certain degree of nuclear atypia may be seen in RD cells

tive in both nodal and extranodal tissues.[4,19,21]

Immunohistochemical Studies. Immunohistochemical studies show that RD cells are strongly and consistently positive for S-100 protein (Fig 2). They also bear markers of macrophage lineage, including HAM56, CD14 (recognized by antibody Leu-M3), and CD68 (EBM11); antigens commonly detected in cells with phagocytic activity, Fc receptor of IgG, and CD16 (Leu-11b); lysosomal enzymes, consistently but not solely present in sinus histiocytes, α1-antitrypsin, α1-antichymotrypsin, and lysozyme; antigens associated with metabolic and functional "activation," including Ki-1 (CD30), transferrin receptor (CD71), and interleukin-2 receptor (CD25); antigens expressed in early stages of inflammation, including Mac 387 and 27E10; and antigens found on monocytes as opposed to tissue macrophages, including PHM3, CD36 (OKM5), and CD15 (Leu-M1).[6,17,23] Cells with these markers have been considered recently immigrated monocytes.[10,24] The plasma cell component is polyclonal, with positive reactivity for both κ and λ light chains.[21]

Immunohistochemistry has given variable, sometimes conflicting, data in detecting CD1a antigen expression on RD cells.[23,25-29] Recently, however, the use of a new monoclonal antibody (MoAb 010) that detects CD1a in routinely processed tissues[30] has revealed a consistent expression of CD1a in RD cells, normal interdigitating reticulum cells, and Langerhans' cells (LCs), but not in macrophages.[5]

The consistent and strong S-100 protein reactivity and the expression of CD1a seem to imply a tenuous relationship of RD cells with normal sinus histiocytes, which are, with few exceptions,[31] S-100 protein–negative and CD1a-negative. Conversely, a possible relationship of RD cells with the LC/IRD cell lineage that expresses both antigens[5] can be suggested.

Rosai-Dorfman cells co-express cathepsins D and E. The co-expression of these markers may be of diagnostic value in distinguishing RD cells from normal skin LCs (cathepsin D–negative and cathepsin E–positive), from proliferating LC-like cells in dermatopathic lymphadenitis (cathepsin D–negative and cathepsin E–negative or –positive), and from normal phagocytes (cathepsin D–positive and cathepsin E–negative).[5]

Although the antigenic profile of RD cells is useful for diagnostic purposes, it does not seem effective in establishing an ontogenetic relationship between RD cells and either professional phagocytes or immune accessory cells.[5,25]

DIFFERENTIAL DIAGNOSIS

Nodal Disease. The differential diagnosis of SHML includes reactive sinus hyperplasia, LC histiocytosis (LCH), Hodgkin's disease (HD), metastatic carcinoma, malignant melanoma, and lymphoma.

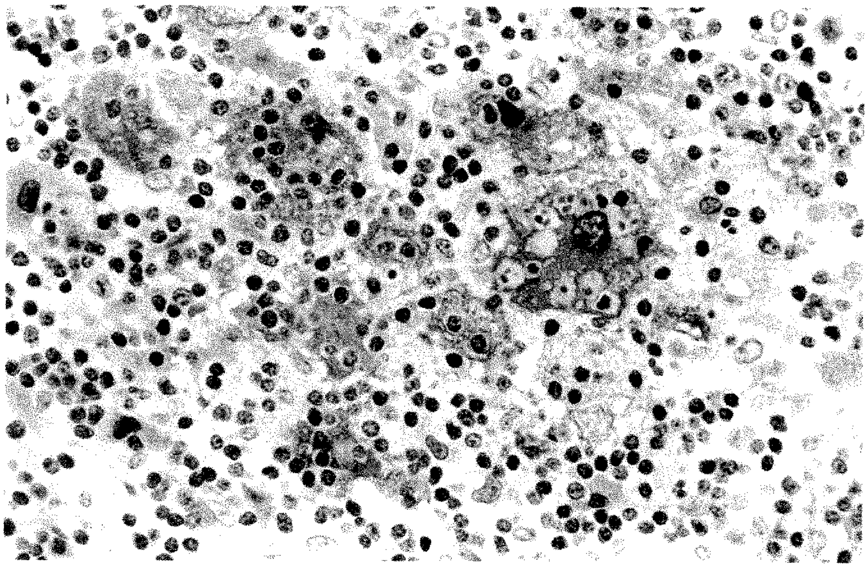

Fig 2. Immunostain for S-100 protein (avidin-biotin complex method, hematoxylin counterstain, original ×400). Histiocytelike cells (Rosai-Dorfman cells) are positive for S-100 protein. Lymphophagocytosis is easy to detect.

By microscopy, reactive sinus hyperplasia may mimic RD disease at low power, but sinus histiocytes differ cytologically from RD cells, do not express S-100 protein, and lack emperipolesis.[11]

Langerhans' cell histiocytosis may also have a low-power microscopic appearance similar to that of RD disease, insofar as nodal sinuses expanded by LCs in LCH produce a light microscopic appearance similar to that of RD disease.

Moreover, the LCs, like the RD cells, are S-100 protein–positive by immunohistochemistry; however, these LCs are different cytologically from RD cells, lack lymphocytophagocytosis, and reveal, on ultrastructural study, the presence of characteristic intracytoplasmic rod-shaped Birbeck granules. In the polymorphous cellular infiltrate accompanying LCs, plasma cells are rare or absent and eosinophils are prominent. Erythrophagocytosis by sinus histiocytes may be present. Foci of necrosis within distended sinuses may be prominent, often surrounded by a rim of eosinophils (so-called eosinophil microabscesses).[6,11]

Lymph nodes affected by HD may have large numbers of foamy macrophages and mimic RD disease microscopically. In nodular sclerosis HD, specific variants of Reed-Sternberg (RS) cells (the lacunar cells) may be present and confused with RD cells. However, RS cells and their variants do not show emperipolesis and are S-100 protein–negative, CD15-positive, and CD30-positive, whereas RD disease cells are S-100 protein–positive and CD15-negative, and CD30 is expressed in RD cells in a weak and scattered way, in contrast to the diffuse and intense reactivity seen in RS cells.[21,32]

It should be noted that RD disease–like changes can sometimes be observed in lymph nodes involved by other processes, such as HD.[33]

Sinusoidal lymph node involvement by metastatic carcinoma, malignant melanoma, and lymphomas, including large-cell anaplastic CD30-positive lymphoma, may assume a morphological appearance similar to that of RD disease, particularly if malignant cells show a propensity to cytophagocytosis. Immunohistochemical findings may be very useful in these settings. Positive staining for pankeratin (ie, MNF-16) strongly supports a diagnosis of carcinoma. S-100 protein is a highly sensitive marker for malignant melanoma, but it lacks specificity; as noted above, the characteristic cells of both RD disease and LCH are positive for S-100 protein as well. Staining with additional antibodies such as HMB45 and NK1/C3 (even though both of them are not entirely specific) is advisable. Leukocyte common antigen (CD45RB) is a specific marker for the hematolymphoid lineage, but lack of staining does not completely exclude the possibility of lymphoma. If lymphoma is suspected, this possibility should be further pursued with B- and T-cell markers, anti-immunoglobulin antibodies, and CD30 antibody.[6]

Extranodal Head and Neck Disease. When the lesion involves the extranodal head and neck area, the differential diagnosis should include rhinoscleroma, fibromatosis, benign fibrous histiocytoma, malignant fibrous histiocytoma, and Wegener's granulomatosis.

Rhinoscleroma is an infectious disease of the upper respiratory tract, uncommon in Western countries, caused by *Klebsiella rhinoscleromatis*. It is characterized by a diffuse and polymorphous cellular infiltrate in which large macrophages with foamy cytoplasm ("Mikulicz cells") are the predominant cell type. Morphologically, it can be differentiated from RD disease because it does not have the "sinusal" pattern and there is no evidence of emperipolesis. The etiologic agent can be traced within macrophages with the Warthin-Starry silver stain.[21]

The abundant collagen deposition, spindled morphology of cells, and inconspicuous emperipolesis can make RD disease with localization to soft tissues difficult to differentiate from fibromatosis occurring in the head and neck.[19] Morphologically, fibromatosis presents more abundant collagen and a relatively acellular appearance, the "sinusal" pattern is not seen, and cells lack emperipolesis and are S-100 protein–negative.[21]

Rosai-Dorfman disease in soft tissue may display a storiform pattern to be differentiated from a fibrohistiocytic lesion. In benign fibrous histiocytoma of soft tissues, the cells have a higher nuclear-to-cytoplasm ratio, hyperchromatic nuclei, a more heterogeneous appearance with xanthoma, and multinucleated and Touton-type giant cells. Malignant fibrous histiocytoma has striking pleomorphic nuclear features, increased nuclear size, more hyperchromatism, and increased mitoses. Inflammatory malignant fibrous histiocytoma is also more anaplastic, with an abundant inflammatory cell infiltrate rich in neutrophils. Some of the tumor cells show erythrophagocytosis and leukocytosis.[21]

Destruction of parenchymal and mesenchymal tissues may sometimes be associated with RD disease. When present, it has to be differentiated from bone and/or soft tissue necrosis, which often accompanies Wegener's granulomatosis. Histologically, the latter sharply contrasts with RD disease. It is characterized by geographic necrosis surrounded by palisaded histiocytes, a granulomatous reaction, and a polymorphous benign inflammatory cell infiltrate with S-100 protein–negative histiocytes and occasional giant cells. Increased titers of antineutrophil cytoplasmic antibodies are crucial to the diagnosis of Wegener's granulomatosis.[21]

DIAGNOSTIC WORKUP

Once a patient is suspected of having SHML, the diagnosis and the extent of the disease have to be established. Biopsy samples taken from the affected areas are very useful in the confirmation of the diagnosis of RD disease. In general, ESHML is pathologically diagnosed, and rarely suspected by clinical evaluation. Patients with ESHML should have a diagnostic workup for immunologic abnormalities, since almost half of patients with SHML with extranodal head and neck manifestations have immune dysfunction.[34] Computed tomography and magnetic resonance imaging, sonography, and radionuclide bone scanning may be helpful in the evaluation of the extranodal sites of involvement.[35]

CLINICAL PRESENTATION

Nodal Disease. The onset of RD disease may be preceded by a nonspecific upper respiratory tract infection characterized by fever and pharyngitis.[4] Some patients may give an additional history of malaise, night sweats, or weight loss.[6]

Approximately 90% of patients at presentation have massive, painless, bilateral cervical lymphadenopathies. Other nodal sites may be involved; in order of frequency, they include axillary, inguinal, paraaortic, and mediastinal lymph nodes, with or without concomitant cervical disease.[11] Conversely, solitary lymph node involvement is unusual.[36]

A mild normochromic or hypochromic microcytic anemia is present in the majority of cases. Patients may have red cell autoantibodies, but severe hemolytic anemia is rare. An increase in the number of peripheral blood neutrophilic cells and lymphopenia are frequently found. The erythrocyte sedimentation rate is usually elevated. Most patients have abnormalities in serum protein levels: polyclonal elevation of IgG is the most common. Lymphocyte and histiocyte functions may be inconsistently abnormal. A reversal of the T4/T8 ratio of circulating lymphocytes has been observed.[4,6,10,11,19,21]

Extranodal Disease. Forty-three percent of patients in the RD disease registry have at least 1 site of extranodal localization.[4]

Virtually all organs in the body can be involved by the disease process. The sites typically involved are skin,[4,37] upper respiratory tract,[38-40] salivary glands,[21] bone,[41,42] central nervous system,[43-45] and soft tissues.[19] Two organs that stand out because of their almost universal sparing by the disorder are the spleen and bone marrow.[10]

If lymph nodes are massively enlarged, the disease may be easily recognized. This is not always the case, since extranodal localization may be the predominant or even the exclusive disease manifestation at presentation.[38,39,46] Generalized lymphadenopathy with widespread extranodal manifestation is uncommon.[7]

The frequency with which extranodal disease is associated with lymphadenopathy varies according to the site of extranodal involvement. The involvement of a major salivary gland is almost always associated with nodal involvement, but only half of the patients with central nervous system localization have nodal disease. Extranodal RD disease may occur before, simultaneously with, or after the lymph node disease (Table 3).[4]

Extranodal Sites of Involvement in Head and Neck. The head and neck area represents one of the most common extranodal sites affected by RD disease. Virtually all head and neck sites may be affected in association with, or independent of, nodal disease, but there is a predilection for the nasal cavity and paranasal sinuses.[4,21,47,48]

The majority of patients present with involvement of more than 1 site. Possible sites involved by disease include the nasal cavity and paranasal sinuses,[4,21,22,39,49,50] major salivary glands (the second most common site of involvement),[21,22,48,51-53] oral cavity (infiltration of the mucosa, tongue, and tonsils),[4,21,46,48] pharynx,[12,48] larynx and trachea,[4,38,54-59] maxillary,[21,46] mandibular, and sphenoidal bones,[21] temporal bone and middle ear,[4,51] infratemporal and pterygoid fossae,[21] meninges,[15,44,60] orbital region,[4,19,21,48,61,62] thyroid gland,[4,48,63] external ear,[20] central nervous system,[4] and skin and soft tissues of the head and neck.[19,25,48,64]

Signs and symptoms vary according to the site of occurrence, and include airway-related problems (partial or complete nasal obstruction, sinusitis, saddle-nose deformity, dyspnea, dysphonia, and stridor),[21,22,38,39,50,56,57] epistaxis,[21,50] dysphagia,[12,48] ophthalmic-related problems (proptosis, ptosis, decreased visual acuity),[62] cranial nerve deficits (paresthesia, palsy, or paralysis),[43] pain and tenderness,[21,48,63] and a slowly growing, painless mass.[19,21]

Patients with multiple sites of involvement present with overlapping signs and symptoms[21] (Table 3).

TABLE 3. REVIEW OF USUAL CLINICAL MANIFESTATIONS OF EXTRANODAL RD DISEASE IN HEAD AND NECK

Site of Involvement	*Clinical Features*
Nasal cavity, paranasal sinuses	One of most common involved sites; there may be either diffuse infiltration of mucosa or formation of polyps; adjacent bone may be eroded; usually described clinically as polyps; commonly associated with other extranodal localizations such as orbit, skin, and oral cavity
Orbit, eyelid	Extraconal orbital soft tissue masses with proptosis are major expressions of orbital disease; bones of orbit may have undergone osteolysis or sclerosis; it may be very painful; enucleation of eyeball may be required, but this does not seem to have any effect on overall prognosis
Ocular globe	Ocular globe involvement is rare, but may be lethal
Oral cavity	Most commonly, palate is affected; usually, oral involvement is associated with other extranodal localizations such as nasal cavity
Tonsils	Tends to be persistent; always associated with cervical adenopathy
Parotid and submandibular glands	Gland involvement may be bilateral; frequently associated with lymph node involvement and extracapsular nodal extension; it may be associated with history of dryness of mouth
Larynx and trachea	Tissue infiltration and edema may cause circumferential narrowing or polypoid appearance; vocal cords may be involved, with impaired mobility; multiple lower respiratory tract lesions may be associated; life-threatening dyspnea or marked stridor may require tracheostomy; no adverse effect on overall prognosis
Skin of head and neck	Dermo-epidermal infiltration presents as nodular, papular, or plaque lesions; in some cases, cutaneous rash is associated; subcutaneous lesions are less common, but involvement may seem to be "panniculitis"
Bones of head and neck	Multiple small lytic lesions in skull with sharply or poorly defined borders are more common than large, mixed, or sclerotic lesions; no predilection for specific site in bone; lesions may be painful; they may represent secondary extension from contiguous soft tissue lesions growing nearby
Temporal bones, including middle ear	Dural-based lesions of middle and posterior fossae may produce erosion of petrous bone; progressive hearing loss may result
Soft tissues of head and neck	This subset of patients seems to be older than most other patients with RD disease; most common site of soft tissue involvement is subcutaneous tissue and is often labeled as "skin" involvement
Thyroid gland	Rare; usually, gland is involved as extension from adjacent lymph node; it presents as painful goiter with mixed echogenic pattern and patient is clinically euthyroid
Central nervous system	Involvement is usually confined to intracranial and intraspinal subarachnoidal, subdural, and epidural spaces; plaquelike lesions on meninges simulate meningioma; edema of adjacent brain may be shown by computed tomography; intraspinal extradural mass may produce paraplegia; surprisingly, central nervous system involvement does not seem to affect overall prognosis

COURSE OF DISEASE AND TREATMENT

Rosai-Dorfman disease is considered to be an indolent, self-limited disease.[4] Extranodal involvement does not seem to be associated with more aggressive disease.[19,21] In many cases, an RD disease patient presents to the physician with a history of lymphadenopathy, a biopsy is performed, and the patient is then lost to follow-up. Such a pattern would suggest that in these patients RD disease has not behaved aggressively. Indeed, most afflicted patients undergo spontaneous remission, with disease manifestations gradually subsiding over several months to years. However, severe morbidity and mortality have been attributed to RD disease. Patients may have a destructive disease, with osseous, cartilaginous, and/or soft tissue erosion and destruction with extension into adjacent anatomic compartments.[38,55] On the basis of the clinical course of the disease, patients can be subdivided into 1) those who undergo a complete and spontaneous remission; 2) those with a chronic course, marked by remission and exacerbations; 3) those with persistent but stable disease; 4) those with progressive disease; and 5) patients who die with disseminated nodal disease and/or involvement of many extranodal organ systems and/or involvement of unusual sites (kidney, lower respiratory tract).[6] However, the role of RD disease in the cause of death is difficult to ascertain. Presumably, patients die either from the effects of disseminated nodal and extranodal disease or from the significant alterations in immune function.[34]

There is no ideal treatment for SHML in the head and neck.[65] Aggressive systemic therapy would appear to have no place in the treatment of localized disease. Conversely, in those patients with extensive, recurrent, or progressive disease, which compromises organ function, radical surgical procedures such as craniotomy,[21] partial maxillectomy,[55] or tracheostomy[38] may be needed. Endoscopic surgery is the optimal treatment for nasal involvement and can ensure an improved airway after operation.[22,66] Surgical treatment may be necessary in those patients who have obstructive symptoms due to nodal or extranodal masses. In one case, the resulting narrowing of the airway was so severe that laryngectomy became necessary.[58]

Treatment protocols should mirror the clinical manifestations. A range of therapeutic modalities may be used, and an ideal therapeutic regimen has yet to be identified. Patients may die secondary to therapeutic maneuvers, eg, intracranial hemorrhage shortly after craniotomy in an attempt to resect an intracranial extension of disease.[21] Adjuvant therapies (chemotherapy and/or radiotherapy) have been used, but the efficacy of these agents does not seem evident so far. A combination of vinca alkaloid, an alkylating agent (vinblastine sulfate, chlorambucil, or cyclophosphamide), and corticosteroids may be of value.[34]

UNFAVORABLE PROGNOSTIC FACTORS

Indicators of poor prognosis include widespread dissemination and involvement of the kidney, liver, and respiratory tract; immunologic abnormalities (autoimmune hemolytic anemia, identification of rheumatoid factor, antinuclear antibodies, positive lupus erythematosus preparations); and association of anemia with neutrophilia, lymphocytopenia, or elevated erythrocyte sedimentation rate.[6]

REFERENCES

1. Rosai J, Dorfman RF. Sinus histiocytosis with massive lymphadenopathy. A newly recognized benign clinicopathologic entity. Arch Pathol 1969;87:63-70.
2. Rosai J, Dorfman RF. Sinus histiocytosis with massive lymphadenopathy: a pseudolymphomatous benign disorder. Analysis of 34 cases. Cancer 1972;30:1174-88.
3. Foucar K, Foucar E. The mononuclear phagocyte and immunoregulatory effector (M-PIRE) system: evolving concepts. Semin Diagn Pathol 1990;7:4-18.
4. Foucar E, Rosai J, Dorfman R. Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease): review of the entity. Semin Diagn Pathol 1990;7:19-73.
5. Paulli M, Feller AC, Boveri E, et al. Cathepsin D and E co-expression in sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease) and Langerhans' histiocytosis: further evidences of a phenotypic overlap between these histiocytic disorders. Virchows Arch A Pathol Anat Histopathol 1994; 424:601-6.
6. Warnke RA, Weiss LM, Chan JKC, Cleary ML, Dorfman RF. Atlas of tumor pathology. Tumor of the lymph nodes and spleen. Third series. Fascicle 14. Washington, DC: AFIP, 1995: 349-60.
7. Wright DH, Richards DB. Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease): report of a case with widespread nodal and extranodal dissemination. Histopathology 1981;5:697-709.
8. Lennert K, Niedorf HR, Blumcke S, Hardmeier T. Lymphadenitis with massive hemophagocytic sinus histiocytosis. Virchows Arch B Cell Pathol 1972;10:14-29.
9. Lampert F, Lennert K. Sinus histiocytosis with massive lymphadenopathy: fifteen new cases. Cancer 1976;37:783-9.
10. Rosai J. Lymph nodes. In: Rosai J, ed. Ackerman's surgical pathology. 8th ed. St Louis, Mo: Mosby, 1995:1694-5.
11. Chang KL, Weiss LM. Histiocytic and dendritic cell proliferation. In: Weiss LM, ed. Pathology of lymph nodes. New York, NY: Churchill Livingstone, 1996:401-3.
12. Bankaci M, Morris RF, Stool SE, Paradise JL. Sinus histiocytosis with massive lymphadenopathy. Report of its occurrence in two siblings with retropharyngeal involvement in both. Ann Otol Rhinol Laryngol 1978;87:327-31.
13. Marsh WL Jr, McCarrick JP, Harlan DH. Sinus histiocytosis with massive lymphadenopathy. Occurrence in identical twins with retroperitoneal disease. Arch Pathol Lab Med 1988; 112:298-301.
14. Maennle DL, Grierson HL, Gnarra DG, Weisenburger DD. Sinus histiocytosis with massive lymphadenopathy: a spectrum of disease associated with immune dysfunction. Pediatr Pathol 1991;11:399-412.
15. Tsang W, Yip TT, Chan JKC. The Rosai-Dorfman disease histiocytes are not infected by Epstein-Barr virus. Histopathology 1994;25:88-90.
16. Levine PH, Jahan N, Murari P, Manak M, Jaffe ES. Detection of human herpesvirus 6 in tissues involved by sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease). J Infect Dis 1992;166:291-5.
17. Rosai J. Sinus histiocytosis with massive lymphadenopathy. Am J Surg Pathol 1991;15:191-2.
18. Humble JG, Jayne WHW, Pulvertoft RJV. Biological interactions between lymphocytes and other cells. Br J Haematol 1980;45:195-200.
19. Montgomery EA, Meis JM, Frizzera G. Rosai-Dorfman disease of soft tissues. Am J Surg Pathol 1992;16:122-9.
20. Tsang WYW, Chan JKC, Ho WK, Yu HC, Chow LTC. Extranodal Rosai-Dorfman disease: an uncommon cause of persistent nodule in the ear. J Laryngol Otol 1992;106:249-51.
21. Wenig BM, Abbondanzo SL, Childers EL, Kapadia SB, Heffner DR. Extranodal sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease) of the head and neck. Hum Pathol 1993;24:483-92.
22. Goodnight JW, Wang MB, Sercarz JA, Fu YS. Extranodal Rosai-Dorfman disease of the head and neck. Laryngoscope 1996;106:253-6.
23. Eisen RN, Buckley PJ, Rosai J. Immunophenotypic characterization of sinus histiocytosis with massive lymphadenopa-

thy (Rosai-Dorfman disease). Semin Diagn Pathol 1990;7:74-82.

24. Van Der Oord JJ, Facchetti F, Delabie J, et al. T lymphocytes in non-neoplastic lymph nodes. In: Guntman E, Vollmer E, eds. Current topics in pathology. Vol 84. Reaction patterns of lymph node. Part I. Cell types and functions. Berlin, Germany: Springer-Verlag, 1990:149-78.

25. Paulli M, Rosso R, Kindl S, et al. Immunophenotypic characterization of the cell infiltrate in five cases of sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease). Hum Pathol 1992;23:647-54.

26. Bonetti F, Chilosi M, Menestrina F, et al. Immunohistological analysis of Rosai-Dorfman histiocytosis. A disease of S-100$^+$, CD1$^-$ histiocytes. Virchows Arch A Pathol Anat Histopathol 1987;411:129-35.

27. Sacchi S, Artusi T, Torelli U, Emilia G. Sinus histiocytosis with massive lymphadenopathy. Leuk Lymphoma 1992;7: 189-94.

28. Lopez P, Estes ML. Immunohistochemical characterization of histiocytes in sinus histiocytosis with massive lymphadenopathy. Analysis of an extranodal case. Hum Pathol 1989;20: 711-5.

29. Andreesen R, Bross KJ, Osterholz J, Emmrich F. Human macrophage maturation and heterogeneity: analysis with a newly generated set of monoclonal antibodies to differentiation antigens. Blood 1986;67:1257-64.

30. Krenács L, Tiszalvicz L, Krenács T, Boumsell L. Immunohistochemical detection of CD1a antigen in formalin-fixed and paraffin-embedded tissue sections with monoclonal antibody 010. J Pathol 1993;171:99-104.

31. Jaffe ES. Histiocytosis of lymph nodes: biology and differential diagnosis. Semin Diagn Pathol 1988;5:376-90.

32. Kapadia SB. Hodgkin's disease. In: Barnes L, ed. Surgical pathology of the head and neck. New York, NY: Marcel Dekker, 1986:1074-90.

33. Falk S, Stutte HJ, Frizzera G. Hodgkin's disease and sinus histiocytosis with massive lymphadenopathy-like changes. Histopathology 1991;19:221-4.

34. Foucar E, Rosai J, Dorfman RF. Sinus histiocytosis with massive lymphadenopathy: an analysis of 14 deaths occurring in a patient registry. Cancer 1984;54:1834-40.

35. McAlister WH, Herman T, Dehner LP. Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease). Pediatr Radiol 1990;20:425-32.

36. Baden E, Caveriviere P, Carbonnel S. Sinus histiocytosis with massive lymphadenopathy (Destombes-Rosai-Dorfman syndrome) occurring as a single enlarged submandibular lymph node: a light and immunohistochemical study with review of the literature. Oral Surg Oral Med Oral Pathol 1987;64:320-6.

37. Thawerani H, Sanchez RL, Rosai J, Dorfman RF. The cutaneous manifestations of sinus histiocytosis with massive lymphadenopathy. Arch Dermatol 1978;114:191-7.

38. Leighton SE, Gallimore AP. Extranodal sinus histiocytosis with massive lymphadenopathy affecting the subglottis and trachea. Histopathology 1994;24:393-4.

39. Gregor RT, Ninnin D. Rosai-Dorfman disease of the paranasal sinuses. J Laryngol Otol 1994;108:152-5.

40. Ngendahayo P, Roels H, Quatacker J, Boddaert J, Ntabomura V, Mbonyingabo P. Sinus histiocytosis with massive lymphadenopathy in Rwanda: report of eight cases with immunohistochemical and ultrastructural studies. Histopathology 1983;7: 49-63.

41. Walker PD, Rosai J, Dorfman RD. The osseous manifestation of sinus histiocytosis with massive lymphadenopathy. Am J Clin Pathol 1981;75:131-9.

42. Sartoris DJ, Resnick D. Osseous involvement in sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease). Eur J Pediatr 1986;145:238-40.

43. Foucar E, Rosai J, Dorfman RF, Brynes RK. The neurologic manifestations of sinus histiocytosis with massive lymphadenopathy. Neurology 1982;32:365-71.

44. Song SK, Schwartz IS, Strauchen JA, et al. Meningeal nodules with features of sinus histiocytosis with massive lymphadenopathy. Am J Surg Pathol 1989;13:406-12.

45. Sanchez R, Rosai J, Dorfman RF. Sinus histiocytosis with massive lymphadenopathy: an analysis of 113 cases with special emphasis on its extranodal manifestations [Abstract]. Lab Invest 1977;36:21.

46. Shemen L, D'Anton M, Klijian A, Toth I, Galantich P. Rosai-Dorfman disease involving the premaxilla. Ann Otol Rhinol Laryngol 1991;100:845-51.

47. Naidu RK, Urken ML, Som PM, Danon A, Biller HF. Extranodal head and neck sinus histiocytosis with massive lymphadenopathy. Otolaryngol Head Neck Surg 1990;102:764-7.

48. Foucar E, Rosai J, Dorfman RF. Sinus histiocytosis with massive lymphadenopathy. Ear, nose, and throat manifestations. Arch Otolaryngol 1978;104:687-93.

49. Sneige N, Batsakis JG. Sinus histiocytosis with massive lymphadenopathy. Ann Otol Rhinol Laryngol 1989;98:241-2.

50. Sheen T-S, Chang Y-L, Yeh K-H, Wu C-T, Ko J-Y. Nasal septal tumor as a sole presentation in the head and neck region in Rosai-Dorfman disease. Otolaryngol Head Neck Surg 1998; 118:408-11.

51. Schweitzer VG, Bobier GD. Sinus histiocytosis with massive cervical lymphadenopathy. Case report and literature review. Ann Otol Rhinol Laryngol 1986;95:331-5.

52. Ünal ÖF, Köybasi S, Kaya S. Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease). Int J Pediatr Otorhinolaryngol 1998;44:173-6.

53. Norman L, Bateman AC, Watters GWR, Singh V, Spedding AV. Rosai-Dorfman disease presenting as a parotid mass. J Laryngol Otol 1997;111:1091-3.

54. Carpenter RJ III, Banks PM, McDonald TJ, Sanderson DR. Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease): report of a case with respiratory tract involvement. Laryngoscope 1978;88:1963-9.

55. Courteney-Harris RG, Goddard MJ. Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease): a rare case of subglottic narrowing. J Laryngol Otol 1992;106:61-2.

56. Biller HF, Pilch BZ. A 51 year old man with upper airway obstruction and lymphadenopathy. N Engl J Med 1981; 305:1572-80.

57. Suarez CR, Zeller WP, Silberman S, Rust G, Messmore H. Sinus histiocytosis with massive lymphadenopathy: remission with chemotherapy. Am J Pediatr Hematol Oncol 1983;5: 235-41.

58. Michaels L. Pathology of the larynx. Berlin, Germany: Springer-Verlag, 1984:329-40.

59. Wenig BM, Devaney K, Wenig BL. Pseudoneoplastic le-

sions of the oropharynx and larynx simulating cancer. Pathol Annu 1995;30:143-87.

60. Carey MP, Case CP. Sinus histiocytosis with massive lymphadenopathy presenting as a meningioma. Neuropathol Appl Neurobiol 1987;13:391-8.

61. Dolman PJ, Harris GJ, Weiland LH. Sinus histiocytosis involving the lacrimal sac and duct. A clinicopathologic case report. Arch Ophthalmol 1991;109:1582-4.

62. Foucar E, Rosai J, Dorfman RF. The ophthalmologic manifestations of sinus histiocytosis with massive lymphadenopathy. Am J Ophthalmol 1979;87:354-67.

63. Larkin DFP, Dervan PA, Munnelly J, Finucane J. Sinus histiocytosis with massive lymphadenopathy simulating subacute thyroiditis. Hum Pathol 1986;17:321-4.

64. Suster S, Cartagena N, Cabello Inchausti B, Robinson MJ. Histiocytic lymphophagocytic panniculitis. An unusual extranodal presentation of sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease). Arch Dermatol 1988; 124:1246-9.

65. Komp DM. The treatment of sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease). Semin Diagn Pathol 1990;7:83-6.

66. Ku PKM, Tong MCF, Leung CY, Pak MW, van Hasselt CA. Nasal manifestation of extranodal Rosai-Dorfman disease — diagnosis and management. J Laryngol Otol 1999;113:275-80.

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PEDIATRIC TYPE I THYROPLASTY: AN EVOLVING PROCEDURE

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The treatment of vocal fold paralysis by type I thyroplasty in the pediatric age group has not been reported. From 1990 to 1998, 12 type I thyroplasty procedures were performed on 8 patients between 2 and 17 years of age. The most common cause of vocal fold paralysis was neurologic, followed by vagal injury from a cardiac procedure. The most common indications for the procedure were aspiration and dysphonia. In our early thyroplasty experience, adult techniques and measurements adapted after Isshiki or Netterville were used. Postoperative laryngoscopy showed that in most cases, the placement of the implant was too high. There were variable outcomes in aspiration and dysphonia with this technique. These findings appear to be independent of thyroplasty approach or of implant design type. We conclude that the standard approach for vocal fold medialization in the adult cannot be applied accurately in the pediatric population. In performing pediatric thyroplasty, the anatomically lower position of the vocal fold must be taken into consideration. We have since modified our technique to adjust for accurate identification of the vocal fold line and medialization. The modified approach for vocal fold medialization in the pediatric population is discussed.

KEY WORDS — aspiration, pediatric thyroplasty, thyroplasty, vocal fold paralysis.

INTRODUCTION

Medialization of the vocal fold for unilateral vocal fold paralysis was popularized by Isshiki et al[1] in 1974. There have been no published series on the applications, indications, and technique of type I thyroplasty in the pediatric population. Since the introduction of thyroplasty techniques, the procedure has been modified and altered by many authors.[2-6] The applications of the original description of type I thyroplasty have extended beyond that of medialization for unilateral vocal fold paralysis and include voice improvement for vocal fold atrophy[7,8] and improving glottic closure in the treatment of aspiration.[9,10] Though the indications have expanded, the basic surgical procedure and placement of the thyroplasty window have not altered much since the original description by Isshiki et al.[1] The success of the operation depends on an understanding of the relationship between the external thyroid cartilage landmarks and the endolaryngeal structures. Correct identification of the level of the vocal fold line with precise placement of the thyroplasty window is necessary for accurate medialization of the true vocal fold. In adults, the level of the vocal fold line is determined by halving the distance between the thyroid cartilage notch and the inferior thyroid cartilage border in the midline. The window is then fashioned by freehand measurements or templates provided by pre-fashioned kits,[3,5] with the superior margin of the window placed at the vocal fold line. The adult thyroplasty window height ranges from 5 to 6 mm, and the window length ranges from 10 to 14 mm, depending on the size of the cartilage and the gender of the patient. The relationships of the endolaryngeal structures to the external thyroid cartilage landmarks have not been determined fully in the pediatric larynx. Because the laryngeal cartilage does not reach adult size until puberty,[11] the application of thyroplasty window measurement guidelines recommended for adults may not be appropriate or transferable to the pediatric patient.[12]

The purpose of this study was to review our operative experience with pediatric type I thyroplasty. After review of our initial pediatric thyroplasty procedures in which adult measurement guidelines were used, we found that in most cases, the thyroplasty window and implant placement were uniformly too high and in the false vocal fold region. This finding prompted a critical assessment of the procedures performed at our institution prior to 1997 with the application of adult techniques to the pediatric patient. We review our experience with pediatric type I thyroplasty and describe our current modification of the surgical procedure to adapt to the size differences in the pediatric larynx.

PATIENTS AND METHODS

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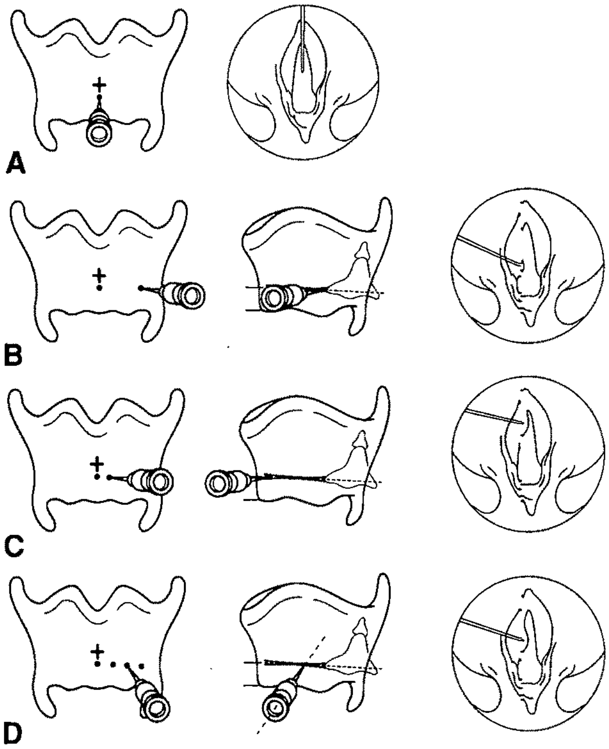

Fig 1. Steps for identification of vocal fold line in pediatric patient. Cross in midline represents distance halfway between thyroid cartilage notch and inferior border. Needle is placed in anterior midline approximately 1 to 2 mm inferior to cross. A) Simultaneous endoscopic visualization identifies anterior commissure. B) Identification of posterior vocal fold landmark and posterior limit of thyroplasty window, which approximates vocal process. C) Identification of anterior limit of thyroplasty window. D) Connecting points between anterior and posterior points along level of vocal fold line.

ter Department of Otolaryngology laryngeal surgery database was searched for all patients who underwent a type I thyroplasty medialization procedure between 1990 and 1997. Charts were reviewed for indication for procedure, cause of vocal fold paralysis, and presence of symptoms of aspiration or dysphonia. All operative reports were reviewed for description of the procedure, and we specifically noted the thyroid cartilage measurements for window placement, window dimensions, and type and design of implant. The operative technique used followed the guidelines recommended for adult thyroplasty.[1,3] The operative reports were also reviewed for anesthetic technique (attended local versus general). All preoperative and postoperative microlaryngoscopic or flexible laryngoscopic photodocumentation of the larynx was reviewed to evaluate vocal fold position. All patients or their families were contacted by telephone or during a clinic visit to assess for subjective improvement in aspiration or dysphonia.

Patients undergoing thyroplasty in 1998 to the time of publication have undergone type I thyroplasty with techniques specifically adapted to the anatomy of the pediatric larynx. The clinical histories of these patients were reviewed for preoperative symptoms and indications for thyroplasty. An additional preoperative assessment of glottic closure and postoperative assessment of symptoms and glottic closure were done. The evolution of the technique is described below.

Modified Pediatric Thyroplasty Technique. Preoperative airway management is determined by the surgeon and is dependent on patient age and cooperation. In most pediatric patients, the procedure is performed under general anesthesia with a tracheotomy. Another option is a laryngeal mask airway. The steps of the operation are as follows.

1. A horizontal skin incision is made at the level of the cricothyroid membrane, and inferior and superior subplatysmal skin flaps are raised and retracted. The strap muscles are divided in the midline.

2. The ipsilateral sternohyoid muscle is retracted laterally. The ipsilateral thyrohyoid muscle is taken down from the thyroid cartilage to provide access to the entire lateral thyroid lamina.

3. Visualization of the larynx is achieved with video endoscopic equipment with a flexible laryngoscope in the awake patient or a rigid 0° telescope passed transorally in the anesthetized patient.

4. A 27-gauge needle is passed through the thyroid cartilage lamina in the midline to determine the level of the vocal fold and anterior commissure. The midpoint of the distance between the thyroid cartilage notch and the inferior thyroid cartilage border is first identified. The level of the anterior commissure typically lies 1 to 2 mm inferior to the midpoint (Fig 1A). This point is tattooed with methylene blue. Please note that the anterior commissure lies at a lower level than in the adult.

5. The distance is measured between the level of the anterior commissure (AC) and the inferior border of the thyroid cartilage (ITC), and is noted as the AC-ITC distance.

6. The vocal process is identified by using the AC-ITC distance and measuring from just anterior to the ipsilateral inferior cornu to a point directly superior. A 27-gauge needle is passed through the thyroid cartilage lamina. This point will be at the vocal process, which is then verified by the endoscopic view (Fig 1B). This point defines the posterior limit of the thyroplasty window. It is tattooed with methylene blue reference.

7. The anterior limit of the window is determined by passing the needle through the thyroid cartilage

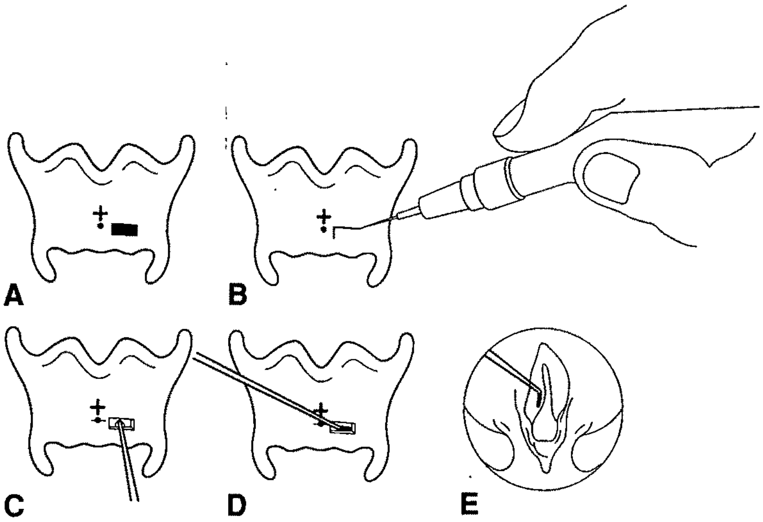

Fig 2. A) Creation of thyroplasty window, measuring height approximately 2 mm below line created for upper edge of true vocal fold. B) Drilling out thyroid cartilage to level of inner perichondrium. C) Elevation of inner perichondrium. D) Pressure point medialization with E) simultaneous endoscopic visualization to determine depth of medialization required.

until a bulge just below the anterior commissure is in approximation with the opposite vocal fold. This point is approximately 3 to 4 mm lateral to the anterior commissure (Fig 1C) and is likewise tattooed with methylene blue.

8. The level of the vocal fold is then mapped out by passing the needle through the cartilage along an imaginary line that connects the anterior and posterior marks (Fig 1D). Care is taken to avoid passing the needle through the fold with each pass of the needle. Rather, the needle is inserted just enough to create a bulge impression at the upper edge of the true vocal fold. This line created will actually be at a slight incline. The remainder of the window can be created at this point.

9. The dimensions of the thyroplasty window are then determined. The height is determined by measuring a distance of 2 mm inferior to the line at the vocal fold. Once this is measured, a parallel line can be drawn with methylene blue 2 mm below the line created, defining the upper edge of the true vocal fold (Fig 2A).

10. A Surgitome dental drill with a 0.8- or 1-mm round drill bit is used to drill out and remove the cartilage in this space, leaving the inner perichondrium intact (Fig 2B).

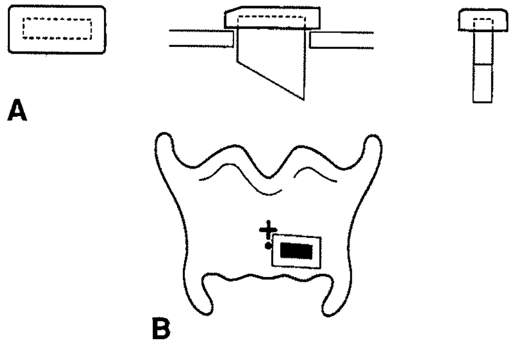

Fig 3. Schematic representation of A) silicone thyroplasty prosthesis design, with B) placement into window.

11. A House round knife is used to elevate the inner perichondrium around the window (Fig 2C). Once the perichondrium is freed around the window, the endoscope is brought into the field to visualize the larynx. An annulus elevator is used to apply pressure to the vocal fold area to determine medialization and whether the window needs to be drilled inferiorly more (Fig 2D). Care should be taken to leave an inferior cartilage strut of 1 to 2 mm or more. If the patient is awake, it is at this point that the determination of medialization depth can be aided by phonation.

12. The annulus elevator is used to determine the anterior and posterior depths of medialization (Fig 2E). Slight overcorrection beyond the midline may be required in patients medialized under general anesthesia.

13. A silicone (extra firm–grade medical) block is fashioned in an umbrella-like prosthesis similar to the one originally described by Isshiki et al.[1] This block is measured and cut to match the inward optimal depth of medialization from the surface of the thyroid cartilage (Fig 3A).

14. The umbrella implant is then placed into the thyroplasty window, and its position is confirmed by endoscopic visualization. It is secured in place in the 4 corners of the window with 4-0 nylon mattress sutures (Fig 3B).

15. The incision is then closed in layers over a Penrose drain.

RESULTS

From 1990 to 1998, 12 type I thyroplasty procedures were performed on 8 patients between 2 and 17 years of age. Patient demographics are described

PATIENT DEMOGRAPHICS

Patient	*Sex*	*Age*	*Indication*	*Cause of VFP*	*TP Side*	*Technique*	*Implant Design*	*Anesthesia*	*Postop Implant Placement*	*Dysphonia*		*Aspiration*	
										Pre	*Post*	*Pre*	*Post*
1	F	17	Dysphonia	Idiopathic	R	Adult-N	Wedge	Attended local	TVF	Yes	No	N/A	N/A
2	F	14	Dysphonia	Congenital	L	Adult-I	Umbrella	Attended local	FVF	Yes	IM	N/A	N/A
3	F	12	Dysphonia, aspiration	CN X injury (cardiac surgery)	L	Adult-N	Wedge	General	FVF	Yes	Yes	Yes	IM
4	F	14	Dysphonia, aspiration	Skull base tumor	R	Adult-N	Wedge	Attended local	FVF	Yes	Yes	Yes	IM
5	F	14	Dysphonia, aspiration	Skull base tumor	R	Adult-N	Wedge	Attended local	FVF	Yes	Yes	Yes	IM
6A*	M	2	Aspiration	Congenital varicella of BS nuclei	L	Adult-N	Wedge	General	FVF	Yes	Yes	Yes	Yes
6B*		3	Aspiration	Varicella	R	Adult-I	Umbrella	General	FVF	Yes	Yes	Yes	IM
6C*		6	Aspiration	Varicella	R	Adult-N	Wedge	General	FVF	Yes	Yes	Yes	Yes
6D*		9	Aspiration	Varicella	R	Ped Mod	Umbrella	General	TVF	Yes	IM	Yes	Yes
7A†	M	7	Aspiration	No VFP	R	Ped Mod	Umbrella	General	TVF	No	No	Yes	No
7B†		7	Aspiration	No VFP	L	Ped Mod	Umbrella	General	TVF				
8	M	2	Dysphonia, aspiration	CN X injury (cardiac surgery)	L	Ped Mod	Umbrella	General	TVF	Yes	No	Yes	No

VFP — vocal fold paralysis; TP — thyroplasty; Adult-N — adult technique with Netterville Laryngoplastic Tray; TVF — true vocal fold; N/A — not applicable; Adult-I — adult technique following Isshiki guidelines; FVF — false vocal fold; IM — improved; CN — cranial nerve; BS — brain stem; Ped Mod — modified pediatric technique.

*Same patient with multiple staged procedures for bilateral thyroplasties for glottic closure.

†Same patient with simultaneous bilateral thyroplasties for glottic closure.

in the Table. The indications for the procedure included aspiration and dysphonia. Six patients with unilateral vocal fold paralysis underwent unilateral type I thyroplasty. One patient with chronic aspiration underwent simultaneous bilateral type I thyroplasties for glottic closure. One with bilateral median vocal fold paralysis from a congenital brain stem varicella infection underwent staged bilateral type I thyroplasty procedures for glottic closure. This patient has required 3 revisions of his right side.

The anesthesia technique was attended local in 4 cases (ages 14 to 17) and general anesthesia via a tracheotomy in 7 (ages 2 to 12).

From 1990 to 1997, thyroplasty was performed with adult measurements and techniques. This was done on 8 vocal folds in 6 patients 2 to 17 years of age. From 1998 to the time of publication, the modified procedure was performed on 4 vocal folds in 3 patients 2 to 7 years of age. In all cases prior to 1997, placement of the thyroplasty window was determined by dividing the height of the thyroid cartilage in half to identify the level of the vocal fold line. The window anterior limit was 4 to 5 mm posterior (lateral) to the midline. The length of the window varied with different ages and ranged from 7 to 13 mm. The window height ranged from 5 to 6 mm and was located generally in the midportion of the cartilage in order to avoid fracturing of the inferior strut. The Netterville Laryngoplastic Tray (Xomed) with a silicone wedge implant design was used in 6 medialization procedures. An Isshiki umbrella-design silicone implant was used in 2 cases. In the cases with adult techniques, postoperative examination of the larynx showed that the thyroplasty implant was too high and was located in the region of the false vocal fold in 7 of the 8 cases.

From 1998 to the time of publication, thyroplasty was performed by the previously described modified technique. The location of the anterior commissure was 1 to 2 mm inferior to the point measured as half of the distance between the thyroid cartilage notch and the inferior border of the thyroid cartilage. The vocal fold line had a slight inclination in the posterior inferior direction in all cases. The anterior limit of the thyroid cartilage window was 3 to 4 mm posterior (lateral) to the midline. The height of the window ranged from 2 mm (patient 8) to 3 mm (patients 7A and 7B). The length varied from 4 mm (patient 8) to 7 mm (patients 6D, 7A, and 7B).

One patient underwent modified pediatric thyroplasty as a revision after 2 previous thyroplasty procedures on the right side (patient 6D). The inferior border of the previously designed window required lowering to correctly identify the vocal fold line. The vocal fold was then medialized by the placement of an umbrella-design modified thyroplasty block to accommodate the dead space from the old window site that was located at the region of the false vocal fold. Postoperative examination of the larynx following the modified pediatric technique showed medialization of the true vocal fold.

Subjective dysphonia was present preoperatively in all cases except the 1 patient in whom the procedure was performed bilaterally for glottic closure. Postoperative dysphonia is still present in 7 of the 8 cases in which adult thyroplasty techniques were used. In these 7 cases, the thyroplasty implant was found to be located in the false vocal fold region. In the 4 cases of medialization by the modified approach, dysphonia has resolved in patient 8 and has improved in patient 6, according to subjective evaluation. This last patient will require revision of his opposite side.

Of the 6 patients with preoperative aspiration, 2 no longer aspirate, 3 are improved, and 1 patient continues to aspirate. This child has required multiple procedures (patient 6) and will need revision thyroplasty on the left side. His procedure is being staged for bilateral glottic closure for chronic aspiration.

DISCUSSION

As the applications of thyroplasty have been extended, so have the age ranges in which the procedure is performed. Applications of thyroplasty in the pediatric patient include not only medialization for unilateral vocal fold paralysis, but also bilateral closure for aspiration. Thyroplasty has the advantage of being a reversible procedure should the need arise or a revision be necessary as the child grows.

We found that the adult measurements advocated by Isshiki et al,[1] Netterville et al,[3] and others are neither easily nor uniformly transferable to pediatric cases. The unique position of the pediatric vocal fold in relation to the thyroid cartilage landmarks, in addition to the smaller dimensions of the pediatric larynx, precludes the use of the standard measurements made in adult patients or provided by prefashioned thyroplasty templates. We found that the level of the fold is not at the midpoint of the anterior thyroid cartilage, as in adults.[1,6,13-15] Our findings are in contrast to a previous report that identified the level of the pediatric vocal fold at one half of the distance between the thyroid notch and the base of the thyroid cartilage.[12] This difference may be attributed to the fact that our findings were determined in vivo, in contrast to the cadaveric dissection of the previous report.[12] Though these differences may seem minor, this lower position of the vocal fold in the pediatric larynx needs to be accounted for during placement of the thyroplasty window, to avoid placement of the implant into the false vocal fold region. The technique of needle guidance allows for accurate determination of the upper edge of the true vocal fold and placement of the thyroplasty window in the pediatric patient. The technique additionally accounts for the very slight slope that the vocal fold takes in the posterior inferior direction as it approaches the vocal process of the arytenoid cartilage. The height of the window will vary, depending on the size of the child and the thickness of the vocal fold. This placement leaves a small margin of thyroid cartilage for an inferior strut. Ideally, a minimum of 2 mm of cartilage should be left between the inferior border of the thyroplasty window and the inferior border of the thyroid cartilage. Care should be taken not to fracture this region. In very young patients, medialization is facilitated by removal of the cartilage. The height of the window in our youngest patients operated on by the modified technique was 2 mm. This height makes it technically challenging to leave the cartilage window in situ. Another advantage of removing the window is that under magnification, one may be able to see the muscle fibers of the vocal fold to aid in implant design and placement. Because the height of the window in very young patients is half the height of the adult window, the wedge implant designs will be difficult to secure without fracturing the thyroid cartilage. The authors prefer the umbrella-type prosthesis similar to the original design of Isshiki et al.[1] This design allows for accurate determination of the depth of medialization from an anterior-to-posterior direction, and the implant can be secured easily to the thyroid cartilage with nylon sutures. Reversal of the thyroplasty is quite easy with this design.

In the evolution of this procedure in younger patients, we did not find that arytenoid adduction was necessary, because of the very short distance between the vocal process of the arytenoid cartilage and the posterior margin of the thyroplasty window in children. We felt that the posterior glottis was adequately medialized. The authors recognize that this concept may need to be revisited as the procedure continues to evolve and we critically look at long-term follow-up of these and subsequent patients.

The choice of general versus attended local anesthesia must be individualized to the patient, and considerations of patient age, maturity, and cooperation should be taken into account. Though we do not have specific guidelines, we feel that the procedure is best done in children under 12 under general anesthesia with the placement of a tracheotomy tube. This allows for visualization and medialization without interference by an endotracheal tube when phonation under attended local anesthesia is not possible. We found that this technique did not interfere with achieving adequate medialization for complete glottic closure. If a thyroplasty is being performed for unilateral vocal fold paralysis, decannulation can proceed once the patient tolerates tracheotomy tube plugging. If, however, the procedure is being performed in a bilateral fashion for glottic closure, decannulation obviously is not a consideration. A laryngeal mask airway is another excellent option for perioperative airway management.

Accurate medialization of the true vocal fold results in improvement or resolution of dysphonia and aspiration. Placement of the thyroplasty implant in the false vocal fold region produces varied outcomes in terms of dysphonia and aspiration. Our initial experience using adult techniques in pediatric thyroplasty reflects this. Only 1 patient in whom adult medialization techniques were used had true vocal fold medialization and resolution of dysphonia (patient 1). The ability to accurately medialize the true vocal fold in this case likely can be attributed to the fact that by the age of 17 years, her larynx was near adult size and the application of adult thyroplasty measurements was transferable. The older the child and the more adult-size the larynx, the higher the likelihood of being able to apply adult techniques to the operation.

The evaluation of the use of preoperative and postoperative assessments of vocal function through stroboscopy and acoustic analysis was not a part of or a goal of this report. We were limited by the retrospective design of the study, as well as by incomplete laryngeal videostroboscopic examinations. It is difficult to discuss the topic of vocal fold medialization without mention of these objective parameters. We found uniformly that the patients who had assessment by laryngeal stroboscopy had results that were often difficult to interpret. This reflects the paucity of reports on the topic of laryngeal stroboscopy in children. As the procedure of vocal fold medialization in the pediatric population becomes more commonly performed, perhaps the analysis of acoustic and stroboscopic parameters will advance as the procedure continues to evolve.

We conclude that vocal fold medialization by a modified type I thyroplasty technique is technically possible in the pediatric population and that there are appropriate indications for the procedure. During the procedure, accurate medialization of the true vocal fold is dependent on precise identification of its level and accurate placement of the thyroplasty window. There is no standard procedure for pediatric patients, and each case must be individualized. We are certain that the operative technique will continue to evolve as we and others increase the number of procedures performed in the pediatric population. Future clinical and research goals will be to evaluate the outcomes of this procedure in children, as well as to determine the timing for revision surgery, if needed, as the larynx grows and develops.

REFERENCES

1. Isshiki N, Morita H, Okamura H, Hiramoto M. Thyroplasty as a new phonosurgical technique. Acta Otolaryngol (Stockh) 1974;78:451-7.

2. Koufman JA. Laryngoplasty for vocal fold medialization: an alternative to Teflon. Laryngoscope 1986;96:726-31.

3. Netterville JL, Stone RE, Luken EJ, Civantos FJ, Ossoff RH. Silastic medialization and arytenoid adduction: the Vanderbilt experience. A review of 116 phonosurgical procedures. Ann Otol Rhinol Laryngol 1993;102:413-24.

4. Montgomery WW, Blaugrund SM, Varvares MA. Thyroplasty: a new approach. Ann Otol Rhinol Laryngol 1993;102: 571-9.

5. Montgomery WW, Montgomery SK. Montgomery® Thyroplasty Implant System. Ann Otol Rhinol Laryngol Suppl 1997; 106(suppl 170).

6. Maragos NE. Type I thyroplasty: pitfalls of modifying the Isshiki approach. How I do it. J Voice 1997;11:470-3.

7. Isshiki N, Kojima H, Shoji K, Hirano S. Vocal fold atrophy and its surgical treatment. Ann Otol Rhinol Laryngol 1996; 105:182-8.

8. Koufman JA. Surgical correction of dysphonia due to bowing of the vocal cords. Ann Otol Rhinol Laryngol 1989;98: 41-5.

9. Flint PW, Purcell LL, Cummings CW. Pathophysiology and indications for medialization thyroplasty in patients with dysphagia and aspiration. Otolaryngol Head Neck Surg 1997; 116:349-54.

10. Pou AM, Carrau RL, Eibling DE, Murry T. Laryngeal framework surgery for the management of aspiration in high vagal lesions. Am J Otolaryngol 1998;19:1-7.

11. Williams PL, Warwick R, eds. Gray's anatomy. Philadelphia, Pa: WB Saunders, 1980.

12. Isaacson G. Extraluminal arytenoid reconstruction: laryngeal framework surgery applied to a pediatric problem. Ann Otol Rhinol Laryngol 1990;99:616-20.

13. Maragos NE. The type I thyroplasty window: implications of normal thyroid cartilage thickness. J Voice 1998;12:107-11.

14. Isshiki N. Recent advances in phonosurgery. Folia Phoniatr 1980;32:119-54.

15. Isshiki N, Taira T, Kojima H, Shoji K. Recent modifications in thyroplasty type I. Ann Otol Rhinol Laryngol 1989;98: 777-9.

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TEN-YEAR EXPERIENCE WITH CERVICAL MINIESOPHAGOSTOMY

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This report describes our experience with 35 patients who underwent intraoperative transcutaneous cervical miniesophagostomy (TCME) during conservation laryngeal and/or hypopharyngeal surgery. The TCME was designed to provide enteral alimentation without the need for a nasogastric tube. Nasogastric tubes may cause posterior laryngeal inflammation, granulations, muscle damage, and vocal cord immobility. Friction between nasogastric and tracheotomy tubes may result in damage to the remaining posterior larynx and may delay healing, oral feeding, and decannulation. Percutaneous endoscopic or radiologically assisted gastrostomy is a possible solution. However, it requires time, special expertise, and coordination with other specialties. In addition, immediate and delayed abdominal complications may occur. The TCME is a relatively simple and quick procedure that is performed during the primary cancer surgery by the head and neck surgeon. It requires no special equipment. It takes about 5 minutes to perform and, if done correctly with tunneling under the skin flaps, is associated with minimal or no postoperative morbidity. It is useful after supraglottic laryngectomy, partial laryngectomy, partial laryngopharyngectomy, and base of tongue resection, and in selected cases of vertical hemilaryngectomy and anterolateral laryngectomy. In the last group, we found that TCME is required if the arytenoid cartilage is removed and the posterior aspect of the larynx is disrupted. There were only minor complications related to TCME. Leakage from the miniesophagostomy did not occur, primarily because of the superior-to-inferior orientation of the tube and the long subplatysmal tunneling before esophageal entrance.

KEY WORDS — enteral alimentation, esophagostomy.

INTRODUCTION

Adequate nutrition is an essential component of perioperative management of head and neck cancer patients. Many patients with malignancy of the upper aerodigestive tract present with a significant nutritional deficit. Since surgical resection often results in additional anatomic and functional deficits that make oral food intake difficult, and since maintaining adequate nutritional status is important for recovery from surgery, we usually rely on alternative methods of enteral alimentation. The nasogastric tube (NGT) has been the standard method of postoperative alimentation in head and neck cancer patients. However, prolonged use of an NGT may lead to nasal necrosis and deformity, sinus infection, and hypopharyngeal or posterior laryngeal irritation, inflammation, ulceration, and granulation.[1-7] In our experience, the coexistence of an NGT and a tracheotomy tube, both being mobile and rigid structures, may result in posterior laryngeal and postcricoid ulceration and granulation. This contributes to postoperative dysphagia and may delay commencement of oral food intake and decannulation.

A gastrostomy tube is certainly a possible solution and has been gaining increasing popularity during the last 15 years.[8-15] The gastrostomy tube may be placed in an open procedure, in a percutaneous endoscopic procedure (PEG), or in a radiologically assisted procedure (RAG). A gastrostomy tube is usually easier to manage and more cosmetically appealing than an NGT. However, gastrostomy tubes may also result in significant complications.[12,16-18] Complications of gastrostomy include abdominal wall infection, intestinal obstruction, wound dehiscence, peritonitis, sepsis, intra-abdominal abscess, and even death.[12,15,18] At our institution, we have witnessed 2 gastrostomy-related deaths in the last 6 years. Both were related to intra-abdominal infectious complications. In addition, at most centers, gastrostomy placement requires a second surgical or radiologic team with additional operating room, endoscopy suite, or radiology suite time.

Gastrostomy remains the best long-term method of postoperative enteral alimentation. However, for the immediate and short-term management of the patient after partial laryngeal resection, we have found the transcutaneous cervical miniesophagostomy (TCME) to be beneficial and advantageous.

PATIENTS AND METHODS

A review of the database of head and neck cancer patients treated by the author at the State University of New York–Health Science Center at Brooklyn and at affiliated hospitals between 1989 and 1998 revealed that 35 patients received intraoperative TCME. The demographic information, site of tumor, surgical procedure, and complications associated with the TCME were recorded.

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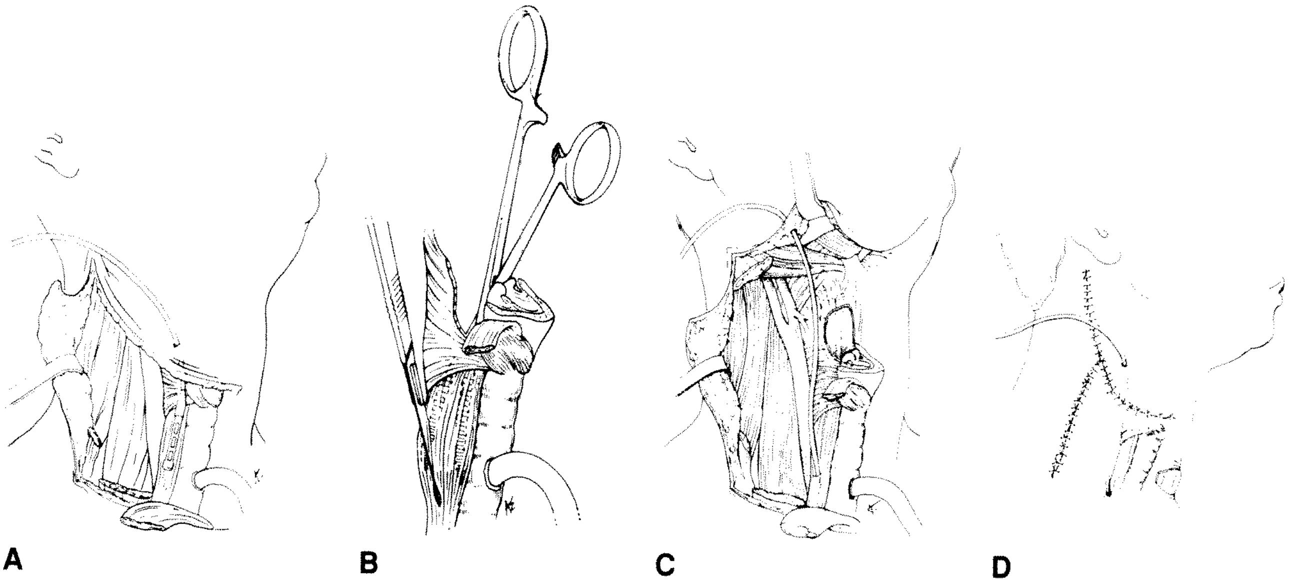

Fig 1. Reprinted with permission.[19] A) 8F feeding tube passed through upper neck flap. B) Esophageal entrance incision. C) Tube passed into distal esophagus. D) Completed transcutaneous cervical miniesophagostomy. Tube is tunneled subcutaneously for 7 cm.

PROCEDURE

The TCME is performed after completion of the resection of the primary tumor and the neck dissection. It is performed before closure or reconstruction of the laryngopharynx. It is usually performed contralateral to the tumor side, preferably on a side with an intact sternocleidomastoid muscle.

The technique itself has not changed much since we introduced it in 1994.[19] The upper neck skin flap is temporarily brought down and redraped (Fig 1A[19]). The skin entrance site is marked, at or just below the level of the angle of the mandible. A stab incision is made, through which an 8F silicone feeding tube is passed into the neck. Next, a long tonsil clamp is placed through the postcricoid region into the esophagus and passed as distally as possible (Fig 1B). The tip of the clamp is used to tent the esophagus, and the bulge is identified in the lower neck, usually at the thoracic inlet level. A No. 11 blade is used to make a stab incision in the esophageal wall onto the tip of the clamp. The tip of the feeding tube is grasped with the jaws of the clamp and passed distally into the esophageal lumen. The clamp is opened and removed, and the feeding tube is passed into the stomach (Fig 1C). Its proper location is verified by injecting air into the tube while the anesthesiologist auscultates the abdomen. Closure and reconstruction of the upper aerodigestive tract are then completed, and the skin is also closed. The result is a feeding tube that enters high in the neck and has a subcutaneous tunnel of at least 7 cm before entering the esophagus (Fig 1D). The feeding tube is secured to the skin at its entrance with a nonabsorbable suture (Fig 2).

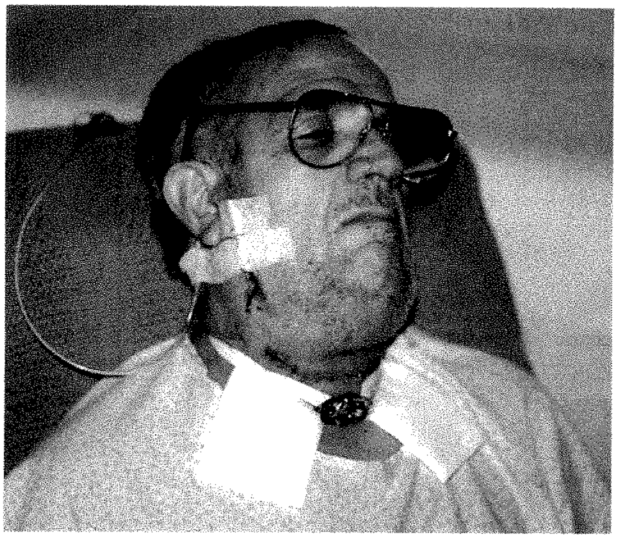

Fig 2. Transcutaneous cervical miniesophagostomy after partial laryngectomy.

Feeding through the TCME starts on the first postoperative day. Once it has been determined that the patient is ready for oral food intake, the TCME feeding tube is removed, a dressing is applied over the skin entrance site, an intravenous line for fluid management is inserted, and the patient is given nothing by mouth for 24 hours. Oral food intake is then resumed.

RESULTS

Thirty-five patients, 26 men and 9 women, ages 38 to 82 years, underwent TCME during a 10-year period. Table 1 shows the site of the primary tumor. Table 2 shows the primary surgical procedures, during which TCME was also performed. Thirty-two of

TABLE 1. SITE OF PRIMARY TUMOR

Site	*No. of Patients*
Supraglottic larynx	11
Glottic larynx	6
Transglottic larynx	3
Hypopharynx	11
Base of tongue	4

TABLE 2. PRIMARY SURGICAL PROCEDURE

Procedure	*No. of Patients*
Supraglottic laryngectomy	11
Vertical hemilaryngectomy	5
Extended, anterolateral, or "three-quarter" laryngectomy	4
Partial pharyngectomy	6
Partial laryngopharyngectomy	5
Base of tongue resection	4

the 35 patients underwent some form of unilateral or bilateral neck dissection. Flap reconstruction was required in 5 of the 35 patients. The TCME was performed by the head and neck surgeon, who devoted 4 to 10 minutes to the procedure.

Thirty-four patients had the TCME tube for 4 to 60 days, with a median of 9 days. One patient who underwent extended supraglottic laryngectomy was not able to regain oral food intake. He was offered a gastrostomy tube, but refused, as he was very comfortable with the TCME and was caring for it on an outpatient basis. Sixteen months after the surgery, he underwent resection of a second primary cancer of the soft palate, during which a gastrostomy tube was placed. Two years later, he died of a third primary cancer in the lung.

Five patients were not able to regain full oral food intake, and their TCME was converted to gastrostomy within 3 to 8 weeks after the original surgery. Of the 7 patients who developed pharyngocutaneous fistula, in 6 patients the fistula occurred on the side contralateral to the TCME. The presence of the TCME had no impact on the management of the fistula, except for the fact that an NGT was not exposed in the depth of the fistula wound. A pharyngocutaneous fistula developed on the side of the TCME in 1 patient who underwent supraglottic laryngectomy with bilateral neck dissection. The feeding tube was exposed in the depth of the fistula in the neck. The fistula developed at the pharyngeal closure site and had no relation to the esophageal entrance of the TCME tube. Aggressive local wound care led to rapid healing without the need to remove the TCME tube.

No major complications related to the placement of the TCME were seen. Three patients had minor complications. One patient who underwent vertical hemilaryngectomy and TCME developed significant postoperative subcutaneous emphysema. However, it is not clear if the emphysema developed secondary to TCME placement or the primary procedure itself. The emphysema resolved within 72 hours without treatment. One patient developed a limited skin infection around the TCME entrance site. This responded to local wound care and antibiotics. The patient who had the TCME for 16 months presented 8 months after surgery with a nonfunctioning, clogged tube. Attempts to clear the tube failed. It was pulled out, and a new tube was immediately inserted without difficulty. Its location was verified radiologically, and the patient resumed his TCME feedings.

It should be noted that during the first 5 years of performing TCMEs, we used this procedure for all hemilaryngectomies. However, we currently do not perform a TCME in a hemilaryngectomy procedure in which all or most of the arytenoid cartilage is preserved and no significant disruption of the posterior larynx is created. This is because healing, decannulation, and resuming oral food intake usually occur very early in the postoperative period. Also, the placement of a TCME during the procedure is more difficult in these patients, because many of them do not require simultaneous neck dissection and the surgical field is very small, usually limited to the larynx only. However, when partial vertical hemilaryngectomy includes complete removal of the arytenoid cartilage and disruption of the posterior larynx, a TCME is of great benefit. In patients who are expected to require a very short period of postoperative enteral alimentation (less than 4 to 5 days) and did not have laryngeal surgery, we continue to use the NGT. We also use the NGT in total laryngectomy patients.

DISCUSSION

Different techniques of cervical esophagostomy have been described in the past.[20-25] Shumrick[26] used the term pyriform sinusotomy. These are usually direct esophagostomies with a higher rate of leakage. Their placement may also be difficult, because of the proximity of the esophagostomy to the reconstructed laryngopharynx. In addition, they usually require surgical closure upon onset of adequate oral food intake. The TCME does not require surgical closure, its entrance into the esophagus is distant from the reconstruction site, and its leakage rate is 0%.

Originally, gastrostomies were used only in patients who required a long-term or permanent method of enteral feeding. However, with the introduction of the relatively simple PEG and RAG, gastrostomies are also used now as temporary procedures. Although gastrostomy avoids the complications of an

TABLE 3. ADVANTAGES OF TRANSCUTANEOUS CERVICAL MINIESOPHAGOSTOMY

Performed by head and neck surgeon
Performed at time of resection
Minimal operative time
Distal to laryngeal and/or pharyngeal suture line
Easy to manage
No leakage
Avoids nasogastric tube nasal complications
Avoids posterior laryngeal irritation
Avoids abdominal complications
Very low complication rate (none major)
Easy to remove, no need for surgical closure

NGT, it introduces a different list of possible complications. Many of these complications are major and may lead to significant morbidity or mortality.[12,15-18] Although a few head and neck surgeons perform PEG,[15] most gastrostomies are performed by gastroenterologists, general surgeons, or interventional radiologists. Therefore, they require a second team with additional anesthetic considerations and operating room, endoscopy suite, or radiology suite time.

The advantages of TCME are shown in Table 3. Ideal candidates for TCME are patients who have had laryngeal and/or pharyngeal surgery in which the entire larynx or part of it was preserved, in whom the surgery included disruption and/or reconstruction at the posterior aspect of the larynx, and who are anticipated to eventually acquire adequate deglutition and oral food intake. Also, preferably, neck dissection was part of the procedure.

REFERENCES

1. Newmark SR, Simpson SM, Beskitt PM, et al. Home tube feeding for long-term nutritional support. J Parenter Enteral Nutr 1981;5:76-8.
2. May M, Nellis KJ. Nasogastric intubation: avoiding complications. Resident Staff Phys 1984;30:60-2.
3. Lipman TO, Kessler T, Arabian A. Nasopulmonary intubation with feeding tubes: case reports and review of literature. J Parenter Enter Nutr 1985;9:618-20.
4. Boscoe MJ, Rosin MD. Finebore enteral feeding and pulmonary aspiration. BMJ 1984;289:1421-2.
5. Miller SK, Tomlinson JR, Sahn SA. Pleuropulmonary complications of enteral tube feedings. Chest 1985;88:230-3.
6. Hafner CD, Wylie JH, Brush BE. Complications of gastrointestinal tract therapy. Arch Surg 1961;83:147-60.
7. Rombeau JL, Barot LR. Enteral nutritional therapy. Surg Clin North Am 1981;61:605-20.
8. Gauderer NW, Ponsky JL, Izant RJ, et al. Gastrostomy without laparotomy. A percutaneous endoscopic technique. J Pediatr Surg 1980;15:872-5.
9. Shike M, Berner YN, Gerdes H, et al. Percutaneous endoscopic gastrostomy and jejunostomy for long-term feeding in patients with cancer of the head and neck. Otolaryngol Head Neck Surg 1989;101:549-54.
10. Hunter JG, Lauretano L, Shellito PC. Percutaneous gastrostomy in head and neck cancer patients. Ann Surg 1989;210: 42-6.
11. Gibson SE, Wenig BL. Percutaneous endoscopic gastrostomy in the management of head and neck carcinoma. Laryngoscope 1992;102:977-80.
12. Gibson SE, Wenig BL, Watkins JL. Complications of percutaneous endoscopic gastrostomy in head and neck cancer patients. Ann Otol Rhinol Laryngol 1992;101:46-50.
13. Bailey CE, Lucas CE, Ledgerwood AM, Jacobs JR. A comparison of gastrostomy techniques in patients with advanced head and neck cancer. Arch Otolaryngol Head Neck Surg 1992; 118:124-6.
14. Selz PA, Santos PM. Percutaneous endoscopic gastrostomy. Arch Otolaryngol Head Neck Surg 1995;121:1249-52.
15. Santos PM, McDonald J. Percutaneous endoscopic gastrostomy: avoiding complications. Otolaryngol Head Neck Surg 1999;120:195-9.
16. Pomerantz MA, Salomon J, Dunn R. Permanent gastrostomy as a solution to some nutritional problems in the elderly. J Am Geriatr Soc 1980;28:104-7.
17. Wilkinson WA, Pickelman J. Feeding gastrostomy: a reappraisal. Am Surg 1982;48:273-5.
18. Wasiljew VJ, Ujiki GT, Beal JM. Feeding gastrostomy: complications and mortality. Am J Surg 1982;143:194-5.
19. Balwally AN, Har-El G. Transcutaneous cervical miniesophagostomy. Otolaryngol Head Neck Surg 1995;113:387-92.
20. Klopp CT. Cervical esophagostomy. J Thorac Cardiovasc Surg 1951;21:490-1.
21. Graham WP III, Royster HP. Simplified cervical esophagostomy for long-term extraoral feeding. Surg Gynecol Obstet 1967;125:127-8.
22. Fitz-Hugh GS, Sly DE. Elective cervical esophagostomy. Ann Otol Rhinol Laryngol 1967;76:804-9.
23. English GM, Morfit HM, Ratzer ER. Cervical esophagostomy in head and neck cancer. Arch Otolaryngol 1970;92:335-9.
24. Dobie RA, Cox KW, Larsen GL. Skin flap esophagostomy: a new procedure. Arch Otolaryngol 1979;105:200-2.
25. Tucker HM, Broniatowski M, Chase S. Tube esophagostomy: a new technique in the management of long-term swallowing disorders. Arch Otolaryngol 1985;111:187-9.
26. Shumrick DA. Pyriformsinusotomy: a useful technique for temporary or permanent tube feeding. Arch Surg 1967;94: 277-9.

ADDENDUM

Since the presentation of this paper, we have performed TCME also with supracricoid laryngectomy, as well as with endoscopic laser supraglottic laryngectomy with neck dissection. For endoscopic supraglottic laryngectomy, a 36F esophageal dilator (bougie) is placed, and the TCME is done through the neck.

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MATURATION OF THE PEDIATRIC TRACHEOSTOMY STOMA: EFFECT ON COMPLICATIONS

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The pediatric tracheostomy stoma can be matured via a technique that places 4-quadrant sutures from the tracheal cartilage to the dermis. This has the potential of decreasing the risk of accidental decannulation and the formation of granulation tissue. A retrospective analysis of 149 tracheostomies performed between January 1989 and December 1996 was done for the following factors: age, underlying diagnosis, indication for tracheostomy, type of tracheal incision, maturation of stoma, duration of tracheostomy, and early and late (>7 days) complications. Maturation of the stoma was performed in 88 (59.1%) of the 149 tracheostomies. There was an overall complication rate of 21.5% (32/149, not including granulation tissue formation). There were 9 (6.0%) early complications and 23 (15.4%) late complications. The overall incidence of tracheocutaneous fistulas occurred in 11 (11.2%) of the 98 decannulated patients: 6 (10.2%) of the 59 matured stomas and 5 (12.8%) of the 39 nonmatured stomas. Granulation tissue was found on subsequent laryngoscopy in 24 (27.3%) of the 88 matured stomas versus 23 (37.7%) of the 61 nonmatured stomas. There were no tracheostomy-related mortalities. Maturing the tracheostomy stoma resulted in a decreased morbidity from accidental decannulations and did not increase the incidence of tracheocutaneous fistulas or granulation tissue formation.

KEY WORDS — complications, incisions, pediatric tracheostomy, stoma maturation.

INTRODUCTION

Historically, tracheostomy in children has been used to relieve upper airway obstruction due to infectious causes (ie, diphtheria, poliomyelitis, epiglottitis). These indications have declined secondary to vaccines and the increasing usage of endotracheal intubations in children. However, the improved survival of critically ill children and preterm infants has resulted in a resurgence of tracheostomies in children. Unfortunately, pediatric tracheostomy continues to be associated with significant morbidity and mortality when compared to adult tracheostomy.[1,2]

The increased morbidity and mortality are more often associated with long-term tracheostomy, but may also occur with short-term tracheostomy. The frequency and severity of complications from pediatric tracheostomy are proportional to the underlying disease and inversely proportional to the child's age.[2,3] This latter trend is most likely due to a maturing pulmonary system in infants and a compliant and actively growing trachea that is easily affected by the introduction of the tracheostomy tube.[4] The type of tracheal incision can have an impact on the growth and patency of the trachea. Controversy surrounds the type of tracheal incision considered appropriate for pediatric tracheostomy.[4,5] The choices include a vertical incision, a horizontal incision, an inferiorly based flap tracheostomy (IBFT), and a superiorly based flap tracheostomy (SBFT). A recent physician survey shows 75% performing a vertical incision, 15% either an IBFT or an SBFT, and 10% using a horizontal incision.[6] Each technique has associated advantages and disadvantages. However, no specific technique exists that decreases the morbidity caused by accidental decannulation while being associated with a low incidence of delayed complications due to tracheocutaneous (TC) fistulas and granulation tissue formation.

At our institution, a technique utilizing a vertical tracheal incision with a 4-quadrant maturation of the tracheostomy stoma has been employed. The purpose of this retrospective study was to present our experience with matured tracheostomies and compare their associated complications against those occurring in nonmatured tracheostomies.

METHODS

Study Design. The medical records of 149 consec-

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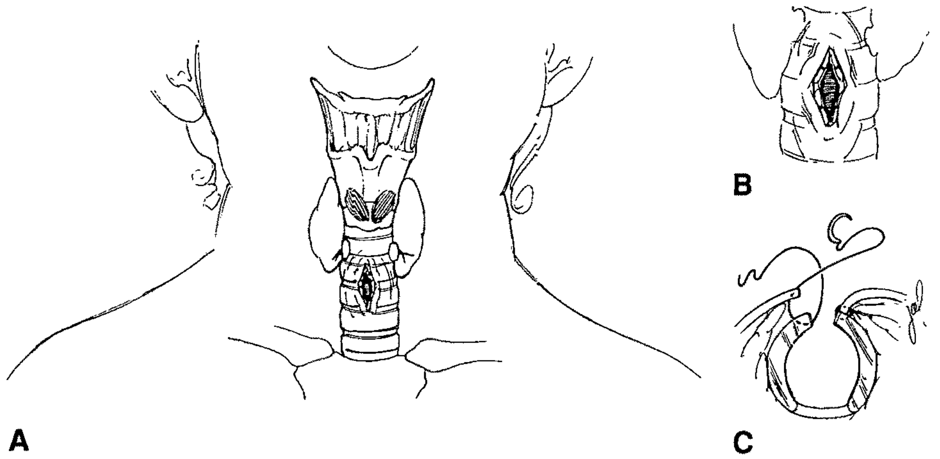

Illustration depicting tracheostomy stoma maturation. A) Frontal view showing matured stoma. B) Closer view of matured stoma. C) Cross-sectional view illustrating placement of maturation suture.

utive tracheostomies performed by the Division of Pediatric Otolaryngology between January 1989 and December 1996 were reviewed. Six patients required more than 1 tracheostomy (2 of whom required 3 separate procedures), making the total patient count 141. A retrospective analysis was done for the following factors: age, underlying diagnosis, indication for tracheostomy, type of tracheal incision, maturation of stoma, duration of tracheostomy, and early and late (>7 days) complications. Data were collected and analyzed with a spreadsheet program (Microsoft Excel). The differences in complications between the matured and nonmatured stoma cases were analyzed with the χ^2 test. A probability level of $p < .05$ was selected as significant.

Procedure. All the procedures were performed under general anesthesia by one of our attending pediatric otolaryngology staff. The tracheostomy was performed as follows with only minor variations, as discussed later. After the placement of proper monitoring instruments, the neck is hyperextended with a shoulder roll. A vertical midline skin incision is made below the level of the cricoid cartilage after infiltration of a local anesthetic agent. Subcutaneous adipose tissue is removed as needed. Cricoid and tracheal rings 1 to 3 are identified. Four-quadrant absorbable maturing sutures are placed from the second and third tracheal cartilages (paramedian) to the dermis of the skin (see Figure, C). A vertical tracheal incision is performed between the maturing sutures (see Figure, A,B). Bilateral nonabsorbable retention sutures are then placed through the cartilage lateral to the incision. With the neck flexed, an appropriate-sized tracheostomy tube is then placed and secured by tracheostomy ties. In select cases, the placement of the tube is verified by rigid bronchoscopy. A postoperative chest x-ray is obtained and evaluated for placement of the tracheostomy tube, pneumothorax, and pneumomediastinum. The patient is observed at least overnight in the intensive care unit, and receives routine tracheostomy care with humidified air and close follow-up by a tracheostomy nurse specialist. The initial tracheostomy tube change is performed at 5 to 7 days postoperatively.

TABLE 1. UNDERLYING DISORDERS (N = 149)

	No.	*%*
Airway obstruction	96	64.4
Subglottic stenosis	29	
Laryngotracheomalacia	24	
Obstructive sleep apnea	18	
Head and neck neoplasm	10	
Vocal cord paralysis	4	
Laryngeal cleft	4	
Subglottic hemangioma	4	
Tracheitis	3	
Central nervous system disorders	38	25.5
Craniofacial disorders	22	14.8
Respiratory disease	19	12.8
Trauma	6	4.0
Cardiac disease	6	4.0

RESULTS

Demographics. There were 88 boys and 61 girls (male-to-female ratio 1.4 to 1.0). The average age at the time of the tracheostomy was 5.6 years (range 3 days to 21 years). Airway obstruction was the most common underlying disorder, followed by central nervous system disorders (Table 1). Subglottic stenosis was the most frequent cause of airway obstruction. The indications for the tracheostomy are listed in Table 2.

TABLE 2. INDICATIONS FOR TRACHEOSTOMY (N = 149)

	No.	*%*
Failure to extubate	76	51.0
Airway obstruction	48	32.2
Airway obstruction after surgery	18	12.1
Aspiration	2	1.3
Respiratory arrest	2	1.3

TABLE 3. TRACHEAL INCISIONS (N = 149)

	Total			
	No.	%	Matured	Nonmatured
Vertical	125	83.9	81	44
Horizontal	9	6.0	4	5
T-shaped	2	1.3	1	1
Cross	1	0.7	0	1
Semicircular	1	0.7	0	1
Unknown	11	7.4	2	9

Matured Versus Nonmatured Stomas. Maturation of the stoma was performed in 88 of the 149 cases (59.1%). The tracheal incisions used are listed on Table 3. The majority of the tracheostomies were performed with a vertical incision. A horizontal incision was done for older children and trauma patients with an average age of 14.6 years. The other incisions were performed in unusual circumstances, including a dog bite to the larynx and a revision tracheostomy after cricothyrotomy.

The average duration of intubation prior to tracheostomy was 16.1 days, and the average duration of postoperative ventilator support was 6.4 days (excluding long-term ventilator-dependent patients). At the time of the chart review, 98 patients were decannulated (65.8%), and the length of time before decannulation ranged from 4 to 1,642 days and averaged 344 days.

Complications. There was a 53.0% (79/149) complication rate in our series (Table 4). The exclusion of granulation tissue formation decreases the complication rate to 21.5% (32/149). There were 9 (6.0%) early complications: accidental decannulation (3), pneumothorax (2), pneumonia (1), subcutaneous emphysema (1), stomal bleeding (1), and stomal stitch breakdown (1). One episode of pneumothorax occurred in a child undergoing concurrent distal tracheal reconstruction for tracheal stenosis. The stoma was matured in this case. The pneumothorax was noted on postoperative chest x-ray, and a chest tube was placed. No accidental decannulation took place. The patient remains cannulated due to persistent tracheal and subglottic stenosis. The other episode of pneumothorax occurred in an 11-year-old child with a nasopharyngeal angiofibroma who had the tracheostomy performed for postoperative airway management. The stoma was not matured, and an accidental decannulation occurred with difficult reinsertion. Pneumothorax resulted, and a chest tube was placed. In contrast, in the 2 other accidental decannulations

TABLE 4. SUMMARY OF COMPLICATIONS

Variables	Categories	Total	Matured Stoma	Nonmatured Stoma	χ^2	p
Cases		149	88/149 (59.1%)	61/149 (40.9%)		
Gender	Male	88	58	30	4.170	.040
	Female	61	30	31		
Age	<1 year old	53/149 (35.6%)	36	17		
	<2 years old	70/149 (47.0%)	48	22		
Decannulation		98/149 (65.8%)	59/88 (67.0%)	39/61 (63.9%)	0.155	.694
Early complications		9/149 (6.0%)	5/88 (5.7%)	4/61 (6.6%)	0.049	.825
	Accidental decannulation	3	2	1		
	Pneumothorax	2	1	1		
	Pneumonia	1	0	1		
	Subcutaneous emphysema	1	0	1		
	Stomal bleeding	1	1	0		
	Stomal stitch breakdown	1	1	0		
Late complications		23/149 (15.4%)	12/88 (13.6%)	11/61 (18.0%)	0.533	.465
	Tracheocutaneous fistula	11/98 (11.2%)	6/59 (10.2%)	5/39 (12.8%)	0.166	.684
	Mucus plug	4	2	2		
	Stomal infection or breakdown	7	4	3		
	Anterior tracheal ulceration from tip	1	0	1		
Granulation tissue		47/149 (31.5%)	24/88 (27.3%)	23/61 (37.7%)	1.810	.178
	Suprastomal	36/149 (24.2%)	18/88 (20.5%)	18/61 (29.5%)	1.610	.204
	Excised	22	13	9		
	Not excised	14	5	9		
	Parastomal	15/149 (10.1%)	6/88 (6.8%)	9/61 (14.8%)	2.500	.113
	Excised	10	4	6		
	Not excised	5	2	3		

incurred by patients with matured tracheostomy stomas, easy reinsertion by the nursing staff occurred without complications.

Stomal bleeding occurred in 1 case and was felt to have been secondary to aggressive suctioning and resolved quickly. Subcutaneous emphysema occurred after a tracheostomy was done for a traumatic laryngeal injury and may have been secondary to the primary injury, but was included for completeness. Pneumonia occurred in a 7-month-old patient with bronchopulmonary disorder–hyaline membrane disease who did not receive perioperative antibiotics.

Late complications (excluding granulation tissue formation) occurred in 23 (15.4%) of the 149 patients: TC fistula (11), mucus plug (4), stomal infection and/or breakdown (7), and anterior tracheal mucosal ulceration (1). The overall incidence of TC fistulas occurred in 11 (11.2%) of the 98 decannulated patients. Of the TC fistulas, 6 (10.2%) occurred in the 59 matured stomas, and 5 (12.8%) in the 39 nonmatured stomas. The difference was not statistically significant ($\chi^2 = 0.166$, $p = .684$). All TC fistulas occurred when the tracheostomy tubes had been in place for more than 1 year (11/25 cases or 44.0%). The average time before decannulation was 609 days for cases with TC fistulas, compared to 344 days for all cases. There were no tracheostomy-related deaths in our series.

Granulation tissue was found on subsequent laryngoscopy in 24 (27.3%) of the 88 matured stomas and in 23 (37.7%) of the 61 nonmatured stomas ($\chi^2 = 1.810$, $p = .178$). Suprastomal granulation tissue was found in 18 (20.5%) of the 88 matured stomas and in 18 (29.5%) of the 61 nonmatured stomas ($\chi^2 = 1.610$, $p = .204$). Parastomal granulation tissues were found in 6 (6.8%) of the 88 matured stomas and in 9 (14.8%) of the 61 nonmatured stomas ($\chi^2 = 2.50$, $p = .113$). None of these showed significant differences, but a trend toward decreased granulation formation with matured stomas is present.

DISCUSSION

There is controversy in the literature about the type of tracheal incision optimal for pediatric tracheostomy.[4,5] Traditionally, vertical tracheal incisions have been utilized in the pediatric population to avoid removal of a tracheal window or flap and to reduce the theoretical risk of tracheal stenosis.[7] In contrast, horizontal incision has been avoided in children due to difficult recannulation and possible suprastomal anterior tracheal wall collapse.[7] It has been suggested, especially for long-term tracheostomies, that the creation of a formal stoma should be considered.[8] This has the potential to decrease the morbidities that can occur after an accidental decannulation and also decrease traumatic recannulation attempts with possible false passage and pneumothorax. A formal stoma will render recannulation easier.

Granulation tissue develops in the raw tissue tract, where the cartilage and tracheostomy tube come into contact. This can occur in up to 80% of tracheostomies and is considered a normal finding by some.[8-10] Exuberant granulation may at times result in tracheal obstruction that can complicate decannulation. When a vertical incision is utilized, the tube may distract the cut cartilages laterally, and the resultant defect above the tube may fill in with granulation tissue (suprastomal granulation). By creating a circumferential mucocutaneous junction at the stoma by the IBFT, SBFT, or maturation technique, the wound complications and granulation tissue formation may be reduced.[8]

Animal studies comparing tracheal incisions present conflicting results. Lulenski and Batsakis[11] compared vertical and inverted U-flap tracheostomies in adult dogs and found that the animals with vertical incisions had a marked decrease in cross-sectional area and lateral diameter. Fry et al[4] utilized different tracheal incisions on weanling ferrets and found that vertical slit incisions caused less tracheal stenosis and less compromise of airflow resistance as compared to the inferiorly based trapdoor and horizontal H incisions. Miller et al[12] compared vertical incisions and SBFTs in developing rabbits and found a 30% decrease in cross-sectional area in the vertical tracheostomy group. It should be noted that this difference was not clinically significant for symptomatic airway obstruction.[12] In addition, these studies did not comment on the incidence of granulation tissue formation.

Rhee et al[13] reviewed 21 SBFTs performed for pediatric long-term tracheostomies. They found a low incidence of complications, with 1 case of wound dehiscence and 1 case of granulation tissue formation. Five patients were decannulated, with 2 (40.0%) requiring a surgical procedure to close the TC fistula. In addition, this technique resulted in a stoma resembling a total laryngectomy stoma and required a substantial increase in procedural time. There was no incidence of tracheal stenosis, but this was a preliminary study with a small number of cases.[13]

Waki et al[9] reviewed 126 IBFTs performed for a pediatric population. Early complications were seen in 4% of patients, and late complications in 50%. (The majority were granulation tissue formation.) They reported a 23% incidence of TC fistula and a 4% incidence of tracheal stenosis that prevented decannulation. Three deaths were attributed to tracheostomy tube obstruction at home.[9]

In comparison, our technique of maturing the stoma with a vertical tracheal incision resulted in an 11.2% incidence of TC fistula. This compared favorably with reported rates in the literature (3% to 43%).[2,9,14,15] The incidence of TC fistula was not increased for cases with a matured stoma as compared to those with a nonmatured stoma. The incidence of granulation tissue formation (31.5%) was comparable to that of other studies.[1,2,10] Maturation of the stoma did not significantly reduce granulation tissue formation, although there was a trend toward a decreased incidence when compared to the cases with a nonmatured stoma. The combination of maturation of the stoma and the use of retention sutures resulted in relatively easy recannulation in the event of accidental decannulation, with the subsequent avoidance of potential complications.

Our technique resulted in no significant incidence of tracheal stenosis that prevented decannulation. Because the pressure on the superior aspect of the tract can lead to collapse of the suprastomal trachea,[8] the maturation of the stoma should add an outward force on the cut edges of the trachea and prevent this collapse. In contrast, IBFT is associated with a 4% incidence of tracheal stenosis.[9] Due to the small number of pediatric cases with SBFT, their incidence of tracheal stenosis has not been determined.[13]

The early complication rate of 6% in our series is comparable to those of 4% to 29% reported in the literature.[1,2,9,10,14] The late complication rate of 47% (including granulation tissue formation) also compared favorably (15% to 53%).[1,2,9,10,14] There was no tracheostomy-related mortality in our series.

CONCLUSION

With the increase in the number of pediatric tracheostomies, the associated morbidity and mortality rates must be minimized. Our technique of utilizing vertical tracheal incisions with maturation of the stoma decreases traumatic recannulation and long-term complications. It provides the benefit of a matured stoma without the increased risk of TC fistulas seen with SBFT and IBFT. Maturing the tracheostomy stoma also results in a trend toward decreased granulation tissue formation. In conjunction with close follow-up, parental education, and home monitoring, stomal maturation can reduce the morbidity and mortality of pediatric tracheostomy.

REFERENCES

1. Crysdale WS, Feldman RI, Naito K. Tracheotomies: a 10-year experience in 319 children. Ann Otol Rhinol Laryngol 1988;97:439-43.
2. Wetmore RF, Handler SD, Potsic WP. Pediatric tracheostomy. Experience during the past decade. Ann Otol Rhinol Laryngol 1982;91:628-32.
3. Sasaki CT, Gaudet PT, Peerless A. Tracheostomy decannulation. Am J Dis Child 1978;132:266-9.
4. Fry TL, Jones RO, Fischer ND, Pillsbury HC. Comparisons of tracheostomy incisions in a pediatric model. Ann Otol Rhinol Laryngol 1985;94:450-3.
5. Hotaling AJ, Robbins WK, Madgy DN, Belenky WM. Pediatric tracheotomy: a review of technique. Am J Otolaryngol 1992;13:115-9.
6. Woolley AL, Muntz HR, Prater D. Physician survey on the care of children with tracheotomies. Am J Otolaryngol 1996; 17:50-3.
7. Kirchner JA. Avoiding problems in tracheostomy. Laryngoscope 1986;96:55-7.
8. Prescott CAJ. Peristomal complications of paediatric tracheostomy. Int J Pediatr Otolaryngol 1992;23:141-9.
9. Waki EY, Madgy DN, Zablocki H, Belenky WM, Hotaling AJ. An analysis of the inferior based tracheal flap for pediatric tracheotomy. Int J Pediatr Otolaryngol 1993;27:47-54.
10. Carter P, Benjamin B. Ten-year review of pediatric tracheotomy. Ann Otol Rhinol Laryngol 1983;92:398-400.
11. Lulenski GC, Batsakis JG. Tracheal incision as a contributing factor to tracheal stenosis. An experimental study. Ann Otol Rhinol Laryngol 1975;84:781-6.
12. Miller FR, Guay ME, Bauer T, Tucker HM. Long-term flap tracheostomy in a pediatric animal model. Arch Otolaryngol Head Neck Surg 1995;121:743-8.
13. Rhee CK, Miller FR, Tucker HM, Eliachar I. The superiorly based flap long-term tracheostomy in pediatric patients. Am J Otolaryngol 1996;17:251-6.
14. Ward RF, Jones J, Carew JF. Current trends in pediatric tracheotomy. Int J Pediatr Otolaryngol 1995;32:233-9.
15. Joseph HT, Jani P, Preece JM, Bailey CM, Evans JN. Paediatric tracheostomy: persistent tracheocutaneous fistula following decannulation. Int J Pediatr Otolaryngol 1991;22:231-6.

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INTRACELLULAR RECORDING FROM POSTERIOR CRICOARYTENOID MOTONEURONS IN THE RAT

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The ability to maintain coordinated vocal cord abduction and upper airway patency is dependent on the integrity of the posterior cricoarytenoid (PCA) motoneurons and their multiple neural connections. Study of the PCA motoneurons represents the initial step in understanding the complex mechanisms responsible for coordinated vocal cord abduction and may provide an insight into the possible pathological processes underlying the various clinical presentations of vocal cord dysfunction. Intracellular recordings were made from 11 PCA motoneurons in Sprague-Dawley rats, which all showed an inspiratory augmenting discharge pattern that is also characteristic of phrenic nerve activity. The resting membrane potential was −56 ± 11 mV. Two PCA motoneurons were injected with Neurobiotin to demonstrate neuronal morphology, which was found to be similar to that obtained by retrograde labeling with cholera toxin B subunit. The technique described for intracellular recording of PCA motoneurons should allow more detailed morphological, electrophysiological, and immunohistochemical information to be obtained, to thereby identify some of the factors responsible for maintaining normal function of the PCA muscle.

KEY WORDS — intracellular recording, posterior cricoarytenoid motoneurons.

INTRODUCTION

Motoneurons driving the respiratory pump muscles are located at various levels along the spinal cord, while those controlling airway patency are located in the brain stem, primarily in the nucleus ambiguus. The ordered firing pattern of these two pools of motoneurons is responsible for the characteristic rhythmic and coordinated pattern of respiratory activity.[1]

Laryngeal motoneurons are located in the nucleus ambiguus of the ventrolateral medulla in close proximity to respiratory bulbospinal neurons, and receive input from an array of interneurons that modify and regulate their activity, thereby controlling the coordinated activity of the laryngeal musculature. The motoneurons responsible for abduction of the vocal cords are those controlling the posterior cricoarytenoid (PCA) muscle, which is the sole abductor of the larynx. Interneurons imparting respiratory control to these motoneurons are responsible for the characteristic phasic inspiratory activity of the PCA muscle, which has been well documented in both animals[2-13] and humans.[14-20]

The complex neuronal connections with laryngeal motoneurons, including those imparting respiratory modulation, are poorly understood, and the specific cause responsible for loss of respiratory control is unknown. In an attempt to better understand the factors responsible for maintaining normal respiratory control of the laryngeal muscles, and gain insight into possible neurophysiological mechanisms underlying laryngeal dysfunction, we established an animal model for intracellular recording from PCA motoneurons.

There have been few intracellular studies reported in the literature looking specifically at laryngeal motoneurons.[21-25] Barillot et al[21] in 1984 identified laryngeal motoneurons in cats following stimulation of the recurrent laryngeal nerve and classified them as inspiratory or expiratory. The response to stimulation of the superior laryngeal nerve was described, and impaled neurons were filled with horseradish peroxidase. In a further study in 1990, Barillot et al[22] reported more detailed electrophysiological data, and in particular, demonstrated the presence of chloride-dependent inhibitory postsynaptic potentials. Takeda and Haji[25] performed a similar study in cats and also demonstrated the effects of thiopental on laryngeal motoneuron activity. Laryngeal motoneurons were recorded intracellularly, stained with horseradish per-

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oxidase, and studied ultrastructurally by Bras et al.[23] Yajima and Hayashi[24] identified laryngeal motoneurons in rats and demonstrated GABA receptor–mediated inhibition.

Previous intracellular studies of laryngeal motoneurons have not attempted to selectively identify motoneurons of individual laryngeal muscles supplied by the recurrent laryngeal nerve. Development of a model to record specifically from PCA motoneurons creates the opportunity to apply existing techniques used to determine neuron morphology, electrophysiology, and histochemistry to the study of PCA motoneurons, and thereby identify some of the factors responsible for maintaining normal laryngeal abductor function.

By means of a rat model with combined exposure of the PCA muscle for focal stimulation and the brain stem for microelectrode insertion, intracellular recordings were made from PCA motoneurons, and impaled neurons were dye-filled to demonstrate morphology.

METHODS

Animal Preparation. Intracellular recordings were obtained from 11 motoneurons in 5 adult Sprague-Dawley rats (300 to 500 g). Anesthesia was induced by intraperitoneal injection of pentobarbital sodium (30 mg/kg) and maintained with additional intravenous doses (3 mg/kg) as required. Cannulae were inserted into the femoral vein and artery for drug administration and arterial blood pressure monitoring, respectively. Rectal temperature was monitored, and a heating blanket and infrared lamp were used to maintain temperature at 37°C to 38°C.

By a dorsal paramedian approach, the scapula was retracted laterally to expose the brachial plexus and thereby allow identification of the roots of the phrenic nerve. A length of phrenic nerve was dissected and the nerve was divided distally and prepared for standard bipolar recording using silver electrodes. Dissection was then carried medial to the common carotid artery to locate the esophagus and trachea. The esophagus was divided and the upper end was reflected rostrally to expose the paired PCA muscles. The dissection was bathed in mineral oil to prevent drying. The marginal mandibular branch of the facial nerve was exposed for bipolar stimulation through a separate cheek incision.

A tracheal cannula was inserted and the rat was paralyzed with pancuronium bromide (1.0 mg/kg) and artificially ventilated with oxygen-enriched air. End-tidal carbon dioxide level and endotracheal pressure were monitored. Additional doses of pancuronium (0.5 mg/kg) were administered as required to maintain the neuromuscular blockade after establishing that there was no response to noxious stimuli.

The preparation was mounted in a stereotaxic frame that enabled fixation of the skull and spinal column, and bilateral pneumothoraces were made to minimize transmitted respiratory movement. A posterior fossa craniectomy was performed together with the removal of a portion of parietal and occipital bones to expose the brain stem. A cervical laminectomy from C1 to C6 was carried out to limit movement of the brain stem during recording. The dura was opened.

Electrophysiology. Phrenic nerve discharge was amplified (bandwidth 100 Hz to 3 kHz), full wave rectified, and integrated (time constant 50 milliseconds). Mapping of the antidromic field was carried out with glass microelectrodes controlled by a PM20H piezo-hybrid manipulator (Bachofer, Germany). The signal was averaged after 20 to 100 sweeps. A CED 1401 data acquisition system (CED, Cambridge, England) was used and data were analyzed and pictorially represented with Spike 2 (CED) and CorelDRAW! (Corel, Canada), respectively.

The location of the caudal border of the facial nucleus was mapped by recording the ipsilateral antidromic field potential obtained by bipolar stimulation of the marginal mandibular branch of the facial nerve to serve as a reference point for rostrocaudal localization of the PCA motoneurons. The PCA motoneurons were mapped by recording the ipsilateral field potential following focal bipolar stimulation (4 to 7 V) of the PCA muscle. The criteria used to confirm antidromic activation of neurons were a fixed latency response in an all-or-none manner, a triphasic (positive-negative-positive) field, and the collision of antidromic spikes with spontaneous discharges. Following preliminary mapping of the extracellular field with microelectrodes containing 3-mol/L sodium chloride (resistance 1MΩ), intracellular recordings were obtained with glass microelectrodes (50 to 80 MΩ) filled with 1-mol/L potassium chloride containing 1% neurobiotin. Neurons were included if the membrane potential was more negative than –40 mV at its most hyperpolarized point, inspiratory activity with a discharge pattern similar to that of the phrenic nerve was recorded, and the criteria for antidromic activation as described above were met. Two neurons were injected with neurobiotin with depolarizing current pulses (4 to 5 nA; 80% duty cycle 3 to 5 minutes).

Immunocytochemistry. Rats were perfused through the left ventricle of the heart with 200 mL of oxygenated tissue culture medium (Sigma Chemical Co, St Louis, Mo; D-8900), followed by 1 L of 4% formaldehyde in 0.1-mol/L phosphate buffer, pH 7.4. The

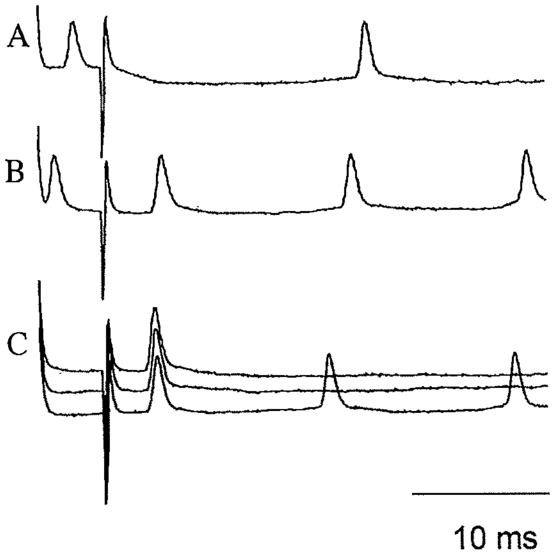


Fig 1. Series of intracellular recordings made from posterior cricoarytenoid (PCA) motoneuron. Recordings A and B show collision of antidromic response with spontaneous spike. Stimulus has been applied within refractory period of spontaneous spike, and antidromic spike is cancelled by action potential traveling orthodromically. Recording C shows series of intracellular recordings displaying typical antidromic response.

brain stem was post-fixed overnight in the same fixative. Serial 50-μm transverse sections were cut through the brain stem and processed to reveal neurobiotin. Sections were incubated for 30 minutes in 10% normal horse serum (NHS) diluted with immunobuffer (IB; 10-mmol/L Tris, 0.9% sodium chloride, 0.05% thimerosal, Sigma Chemical Co, St Louis, Mo: T-5125, in 10-mmol/L sodium phosphate buffer containing 0.3% Triton X-100) and then in ExtrAvidin–horseradish peroxidase (1:500, Sigma Chemical Co) in IB containing 10% NHS for 24 hours. Neurobiotin was revealed in the filled neurons by a nickel-intensified diaminobenzidine reaction with peroxide being generated by glucose oxidase (Llewellyn-Smith et al[26]). All incubations were carried out at room temperature with gentle agitation, and sections were washed in 3 changes of IB after each incubation. Sections were mounted onto glass slides, dehydrated, and coverslipped in DePeX for analysis by light microscopy and color photography. Analog images were scanned (Kodak Slide Scanner) and saved as tagged image file format (TIFF) files. Small changes were made in brightness and contrast by Adobe Photoshop version 3.0. Reconstructions were made from serial axial sections.

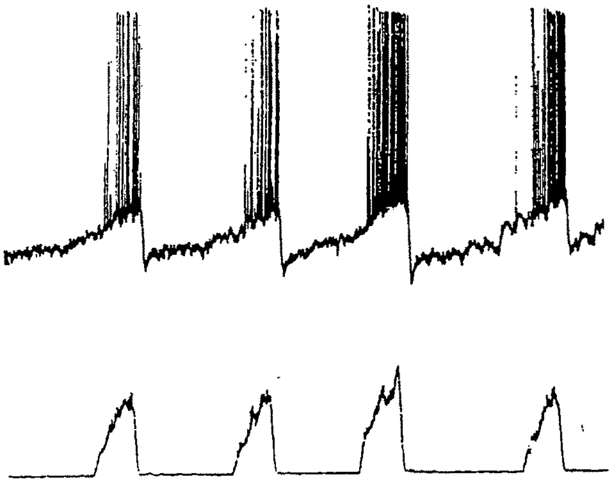


Fig 2. Rhythmic changes in membrane potential of PCA motoneuron shown with phrenic nerve discharge.

DIMENSIONS AND LOCATION OF TWO DYE-FILLED PCA MOTONEURONS

		Location (mm)			
	Dimensions in Length × Width (μm)	*Caudal*	*Lateral*	*Depth*	*Micromanipulator Depth*
Neuron 1	40 × 25	1.45	1.50	1.95	2.02
Neuron 2	30 × 20	0.85	1.40	1.90	2.14

"Micromanipulator depth" is measurement made using micromanipulator. All other measurements taken from histologic section. "Caudal" is distance caudal to caudal pole of facial nucleus, "lateral" is distance from midline, and "depth" is distance from caudal surface of brain stem.

PCA — posterior cricoarytenoid.

The animal experimentation in this study was approved by the Animal Ethics Committee, Royal North Shore Hospital, St Leonards, New South Wales.

RESULTS

Eleven PCA motoneurons were identified by their antidromic activation, and an example of an intracellular recording is shown in Fig 1. The resting membrane potential was –56 ± 11.2 mV (mean ± standard deviation) and ranged from –44 to –78 mV.

Depolarization at the onset of phrenic nerve activity was rapid. All 11 neurons displayed a typical augmenting discharge pattern with depolarization continuing to evolve toward less negative values during the entire inspiratory phase, and rapid repolarization during the period of declining phrenic nerve activity. An example of the rhythmic changes in membrane potential of a PCA motoneuron is displayed in Fig 2.

Two neurons were injected with neurobiotin, and their details are listed in the Table. Photomicrographs of a Neurobiotin-filled neuron are shown in Figs 3 and 4, and a reconstruction is shown in Fig 5.

DISCUSSION

The PCA motoneurons recorded intracellularly following focal stimulation of the PCA muscle all

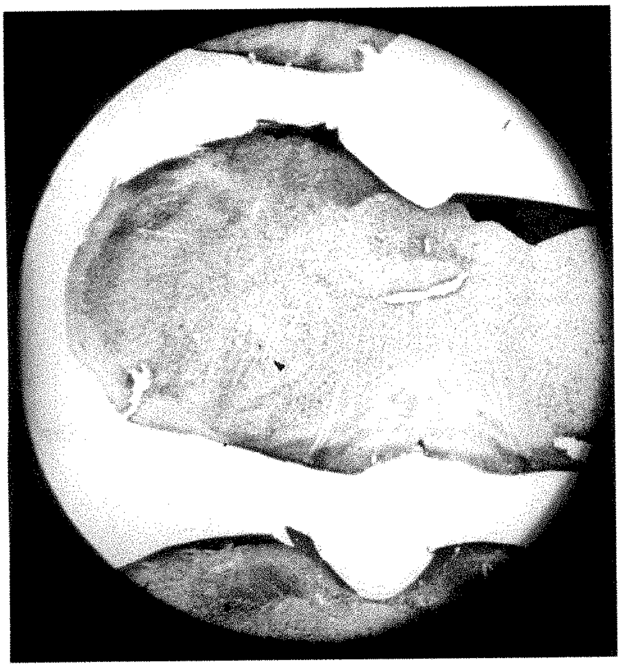


Fig 3. Low-power photomicrograph of rat medulla showing neurobiotin-filled PCA motoneuron (arrowhead).

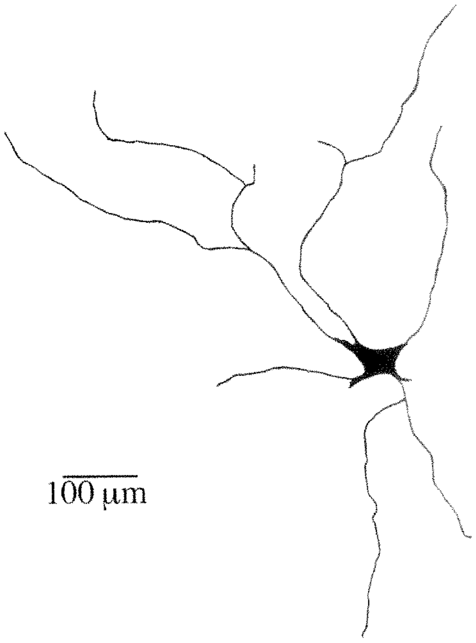
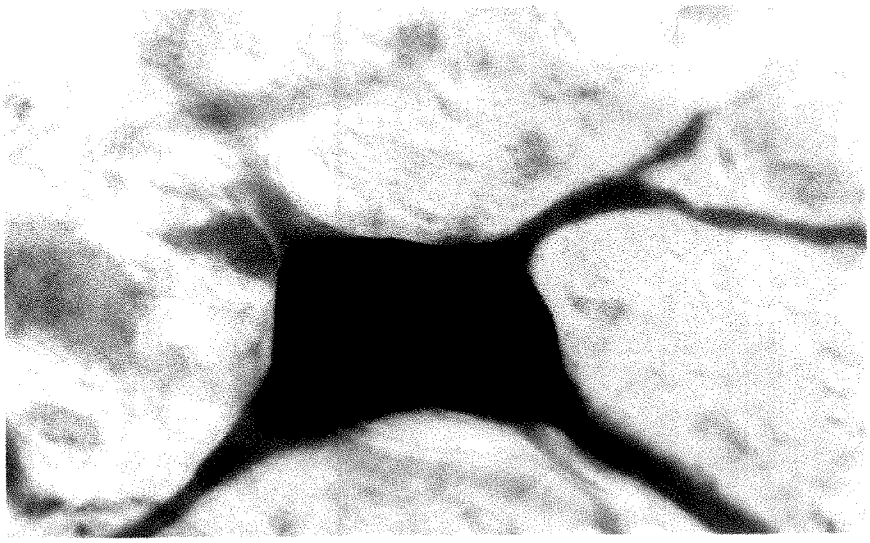


Fig 5. Reconstruction of PCA motoneuron.

showed a typical inspiratory augmenting discharge pattern, which is also characteristic of phrenic nerve activity.[27] In a similar study in cats, Barillot et al[22] identified 16 inspiratory laryngeal motoneurons following recurrent laryngeal nerve stimulation. Nine displayed an augmenting discharge pattern, while in the other 7, maximal depolarization occurred before the peak of phrenic nerve discharge, giving a bell-shaped pattern of depolarization. The membrane rapidly repolarized during the period of declining phrenic activity in all but 2 neurons. The 9 inspiratory augmenting neurons in the study by Barillot et al[22] displayed a discharge pattern similar to that found in PCA motoneurons in this study, and appear to have been PCA motoneurons. The other neurons are likely to be motoneurons of the other laryngeal muscles supplied by the recurrent laryngeal nerve that display inspiratory activity.

Previous reports of laryngeal motoneuron morphology based on retrograde labeling studies have shown little uniformity. In particular, descriptions of PCA motoneuron size in the rat[28-30] differ substantially. The 2 neurobiotin-filled PCA motoneurons in this study were comparable in size and shape with the information obtained following retrograde labeling with cholera toxin B subunit in our previously reported work.[31] Similarities also exist with inspiratory neurons of the dorsal respiratory group in cats, which are predominantly bulbospinal. Bianchi et al[1] have reported a mean somal diameter of 30 to 38 µm with 4 to 10 primary dendrites oriented along the

Fig 4. High-power photomicrograph of neurobiotin-filled PCA motoneuron.



longitudinal axis, and also noted the morphological similarity between laryngeal motoneurons and bulbospinal neurons. Barillot et al[21] identified laryngeal motoneurons intracellularly in cats and described an almost spherical soma with 5 to 10 dendritic trunks and profuse dendritic branching.

While this study has been limited to the development of a technique for identification of PCA motoneurons to obtain basic morphological and electrophysiological information, the technique can be applied to the specific study of PCA motoneurons in greater detail, employing methods previously described for investigation of laryngeal motoneurons in general. Intracellular studies of laryngeal motoneurons have reported the response to superior laryngeal nerve stimulation,[21] effects of negative current injection on membrane potential,[22,25] outcome following administration of thiopental,[25] quantitative ultrastructural information regarding synaptic boutons located on the soma and at various sites along the dendrites,[23] and demonstration of GABA sensitivity by microiontophoretic application of GABAergic drugs.[24] Laryngeal motoneurons have been recorded extracellularly, and their sensitivity to serotonin has been demonstrated by topical application.[32] Double-labeling techniques have been used to demonstrate serotonin-containing[33,34] and substance P–containing[33] immunoreactive fibers surrounding retrogradely labeled PCA motoneurons.

Previous intracellular studies of laryngeal motoneurons have not attempted to selectively identify motoneurons of individual laryngeal muscles supplied by the recurrent laryngeal nerve. By means of the dorsal approach in the rat model for combined exposure of the PCA muscle for focal stimulation and the brain stem for intracellular recording, a wide range of morphological, electrophysiological, and immunohistochemical information can now be obtained regarding PCA motoneurons in experimental animals to document the characteristic mechanisms responsible for maintaining normal function of these motoneurons.

REFERENCES

1. Bianchi AL, Denavit-Saubie M, Champagnat J. Central control of breathing in mammals: neuronal circuitry, membrane properties, and neurotransmitters. Physiol Rev 1995;75:1-45.

2. Murakami Y, Kirchner JA. Respiratory movements of the vocal cords. An electromyographic study in the cat. Laryngoscope 1972;82:454-67.

3. Suzuki M, Kirchner JA. The posterior cricoarytenoid as an inspiratory muscle. Ann Otol Rhinol Laryngol 1969;78:849-64.

4. Sherrey JH, Megirian D. Respiratory EMG activity of the posterior cricoarytenoid, cricothyroid and diaphragm muscles during sleep. Respir Physiol 1980;39:355-65.

5. Orem J, Lydic R. Upper airway function during sleep and wakefulness: experimental studies on normal and anesthetized cats. Sleep 1978;1:49-68.

6. England SJ, Kent G, Stogryn HAF. Laryngeal muscle and diaphragm activities in conscious dog pups. Respir Physiol 1985;60:95-108.

7. Harding R, England SJ, Stradling JR, Kozar LF, Phillipson EA. Respiratory activity of laryngeal muscles in awake and sleeping dogs. Respir Physiol 1986;66:315-26.

8. Bartlett D. Effects of hypercapnia and hypoxia on laryngeal resistance to airflow. Respir Physiol 1979;37:293-302.

9. Mu L, Yang S. The role of the posterior cricoarytenoid muscle in phonation: an electromyographic investigation in dogs. Laryngoscope 1991;101:849-54.

10. van Lunteren E, Van de Graaff WB, Parker DM, et al. Activity of upper airway muscles during augmented breaths. Respir Physiol 1983;53:87-98.

11. van Lunteren E, Van de Graaff WB, Parker DM, et al. Nasal and laryngeal reflex responses to negative upper airway pressure. J Appl Physiol Respirat Environ Exercise Physiol 1984;56:746-52.

12. Scott SC, Inman JD, Moss IR. Ontogeny of sleep/wake and cardiorespiratory behavior in unanesthetized piglets. Respir Physiol 1990;80:83-102.

13. Praud JP, Kianicka I, Leroux JF, Dalle D. Laryngeal response to hypoxia in awake lambs during the first postnatal days. Pediatr Res 1995;37:482-8.

14. Brancatisano TP, Dodd DS, Engel LA. Respiratory activity of posterior cricoarytenoid muscle and vocal cords in humans. J Appl Physiol Respirat Environ Exercise Physiol 1984;57:1143-9.

15. Eichenwald EC, Howell RG, Kosch PC, Ungarelli RA, Lindsey J, Stark AR. Developmental changes in sequential activation of laryngeal abductor muscle and diaphragm in infants. J Appl Physiol 1992;73:1425-31.

16. Duara S, Rojas M, Claure N. Upper airway stability and respiratory muscle activity during inspiratory loading in full-term neonates. J Appl Physiol 1994;77:37-42.

17. Duara S, Neto GS, Claure N. Role of respiratory muscles in upper airway narrowing induced by inspiratory loading in preterm infants. J Appl Physiol 1994;77:30-6.

18. Kuna ST, Smickley JS, Insalaco G. Posterior cricoarytenoid muscle activity during wakefulness and sleep in normal adults. J Appl Physiol 1990;68:1746-54.

19. Kuna ST, Day RA, Insalaco G, Villeponteaux RD. Posterior cricoarytenoid activity in normal adults during involuntary and voluntary hyperventilation. J Appl Physiol 1991;70:1337-85.

20. Kosch PC, Hutchison AA, Wozniak JA, Carlo WA, Stark AR. Posterior cricoarytenoid and diaphragm activities during tidal breathing in neonates. J Appl Physiol 1988;64:1968-78.

21. Barillot JC, Bianchi AL, Gogan N. Laryngeal respiratory motoneurons: morphology and electrophysiological evidence of separate sites for excitatory and inhibitory synaptic inputs. Neurosci Lett 1984;47:107-12.

22. Barillot JC, Grelot L, Reddad S, Bianchi AL. Discharge

patterns of laryngeal motoneurons in the cat: an intracellular study. Brain Res 1990;509:99-106.

23. Bras H, Destombes J, Gogan P, Tyc-Dumont S. The dendrites of single brain-stem motoneurons intracellularly labelled with horseradish peroxidase in the cat. An ultrastructural analysis of the synaptic covering and the microenvironment. Neuroscience 1987;22:971-81.

24. Yajima Y, Hayashi Y. GABA(A) receptor–mediated inhibition in the nucleus ambiguus motoneuron. Neuroscience 1997;79:1079-88.

25. Takeda R, Haji A. Electrophysiological properties of respiratory laryngeal motoneurons and effects of thiopental. Tohoku J Exp Med 1988;156(suppl):21-31.

26. Llewellyn-Smith IJ, Minson JB, Pilowsky PM. Retrograde tracing with cholera toxin B–gold or immunocytochemically-detected cholera toxin B in the central nervous system. In: Conn PM, ed. Neurotoxins. Methods in neuroscience. Vol 8. New York, NY: Academic Press, 1992:180-201.

27. Cohen MI. Neurogenesis of respiratory rhythm in the mammal. Physiol Rev 1979;59:1105-73.

28. Hinrichsen CF, Ryan AT. Localization of laryngeal motoneurons in the rat: morphologic evidence for dual innervation? Exp Neurol 1981;74:341-55.

29. Portillo F, Pasaro R. Location of motoneurons supplying the intrinsic laryngeal muscles of rats. Horseradish peroxidase and fluorescence-labeling study. Brain Behav Evol 1988;32:220-5.

30. Fay R, Gilbert KA, Lydic R. Pontomedullary neurons transsynaptically labeled by laryngeal pseudorabies virus. Neuroreport 1993;5:141-4.

31. Berkowitz RG, Sun Q-J, Chalmers J, Pilowsky P. Identification of posterior cricoarytenoid motoneurons in the rat. Ann Otol Rhinol Laryngol 1999;108:1033-41.

32. Fenik V, Kubin L, Okabe S, Pack AI, Davies RO. Differential sensitivity of laryngeal and pharyngeal motoneurons to iontophoretic application of serotonin. Neuroscience 1997;81:873-85.

33. Holtman JR Jr. Immunohistochemical localization of serotonin- and substance P–containing fibers around respiratory muscle motoneurons in the nucleus ambiguus of the cat. Neuroscience 1988;26:169-78.

34. Arita H, Sakamoto M, Hirokawa Y, Okado N. Serotonin innervation patterns differ among the various medullary motoneuronal groups involved in upper airway control. Exp Brain Res 1993;95:100-10.

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CRICOTHYROID SUBLUXATION: A NEW INNOVATION FOR ENHANCING THE VOICE WITH LARYNGOPLASTIC PHONOSURGERY

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Laryngoplastic phonosurgery has evolved to become a dominant treatment modality for paralytic dysphonia. Current surgical procedures have addressed primarily the position of the musculomembranous vocal fold and the arytenoid in the axial and vertical planes. However, dynamic range capabilities and vocal flexibility have been limited secondary to the flaccid, denervated vocal fold tissue. Therefore, a new procedure was conceived to enhance the acoustic vocal outcome from operations that reposition the vocal edge. Cricothyroid (CT) subluxation was designed as a technique to increase the distance between the cricoarytenoid joint and the insertion of the anterior commissure ligament. Cricothyroid subluxation was done without complication in 9 patients who underwent combined adduction arytenopexy and medialization laryngoplasty, and in 4 patients with medialization laryngoplasty alone. Postoperative stroboscopic assessment was done in all of the 13 patients, while complete analysis of vocal function was available in 10 of the 13 patients; this revealed improvement (as a group) on almost all objective measures over the preoperative state. All patients who underwent CT subluxation had a normal maximum frequency range (pitch variation of more than 2 octaves), as compared with 22% of a prior similar cohort of patients who did not undergo CT subluxation. All patients who underwent CT subluxation had normal glottal airflow and a normal noise-to-harmonics ratio. Cricothyroid subluxation is a relatively easily adjustable procedure that increases the length and viscoelastic tension of the denervated vocal fold. The modified biomechanical properties resulted in improved vocal outcome in all of our patients, which was most remarkable in terms of maximal range capabilities. Cricothyroid subluxation enhanced the postoperative voice of patients regardless of whether they required medialization laryngoplasty alone or whether they also required adduction arytenopexy.

KEY WORDS — adduction, arytenoid, arytenopexy, cricothyroid joint, dysphonia, Gore-Tex, laryngoplasty, medialization, paralysis, phonosurgery, vocal cord, vocal fold, voice.

INTRODUCTION

Laryngoplastic phonosurgery has emerged as the primary surgical intervention for the treatment of paralytic dysphonia. Medialization of the musculomembranous vocal fold by means of reconforming the laryngeal cartilage framework was described by Payr[1] in 1915. This technique was explored further by Meurman,[2,3] Opheim,[4] Waltner,[4] Miehlke,[4] and Sawashima et al[5] in the middle part of this century. The landmark investigations of Isshiki et al[6,7] in the 1970s established a systematic design and organization for phonosurgical alteration of the laryngeal framework.[8] They described a medialization procedure of the musculomembranous vocal fold with the use of a synthetic implant in 1974[6] and, shortly thereafter, devised the arytenoid adduction[7] procedure to treat patients with large glottal gaps secondary to a malpositioned arytenoid. These reports catalyzed the current paradigm shift from injection techniques to laryngoplastic phonosurgery for the treatment of vocal fold paralysis.

Patients who require repositioning of the arytenoid typically have minimal or unfavorable synkinetic reinnervation of the intrinsic laryngeal musculature. Isshiki's classic arytenoid adduction procedure mimics contraction of the lateral cricoarytenoid muscle and achieves rotation of the arytenoid by means of a suture that is placed through its muscular process and directed anteriorly through the thyroid lamina. However, the agonist-antagonist adductor function of the other intrinsic muscles (lateral thyroarytenoid, interarytenoid, and posterior cricoarytenoid) is not simulated. Therefore, Zeitels et al[9] designed the adduction arytenopexy procedure to model the synchronous function of the aforementioned musculature. In this technique, the arytenoid is positioned on the medial aspect of the cricoid facet, simulating its normal adduction during phonation. An implant is then placed lateral to the paraglottic muscles (inner thyroid perichondrium) to prevent wide excursion of the glottal tissues during entrained oscillation. The denervated musculature of the paraglottic space is highly susceptible to the potential closing forces, secondary to Bernoulli's effect. This fact is utilized to counterbalance the loss of normal elastic recoil closing forces caused

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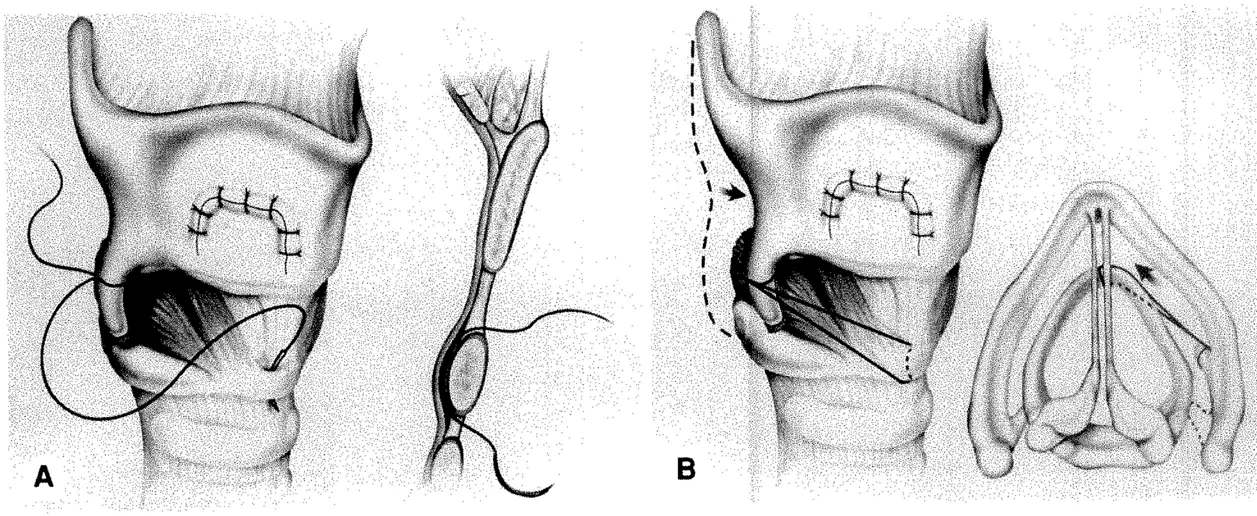

Fig 1. Procedure. A) 2-0 Prolene suture is affixed to previously mobilized inferior cornu of thyroid lamina. It is then passed under anterior cricoid in submucosal fashion. B) Suture is tightened while patient performs number of phonatory gestures. Vector force of cricothyroid subluxation suture models cricothyroid contraction. (Reprinted with permission.[10])

by the denervation. Placing the arytenoid in appropriate position posteriorly, prior to the placement of an implant, achieves most of the medialization of the vocal edge that is necessary for the musculomembranous vocal fold.[9,10]

Observations made from the vocal outcome data in patients who underwent adduction arytenopexy and medialization laryngoplasty revealed that fairly normal conversational-level phonation was achieved.[9] However, there were remarkable limitations in maximal range capabilities, especially in frequency variation and maximal phonation time. This was thought to be secondary to suboptimal viscoelastic tension in the denervated vocal fold soft tissues, despite the aforementioned improvements in 3-dimensional repositioning of the vocal edge. The need to increase viscoelastic tension in the denervated vocal fold, and thereby permit aerodynamically efficient entrained oscillation, catalyzed the development of the cricothyroid (CT) subluxation procedure.

MATERIALS AND METHODS

Operative Technique. The CT subluxation procedure is done subsequent to adduction arytenopexy and/or medialization laryngoplasty.[9,10] A medialization laryngoplasty is done without an adduction arytenopexy if there is favorable synkinetic reinnervation that results in a well-positioned arytenoid. The operative technique for CT subluxation has been described in detail,[10] so that only the highlights of the procedure will be delineated. Unless it is contraindicated, patients are given 0.2 mg/kg of Decadron 90 minutes prior to the procedure. We believe that this helps to minimize intraoperative swelling, which can alter judgment regarding the implant size for medialization and the tension of the CT subluxation suture. A double-pronged skin hook is placed lateral to the edge of the thyroid lamina, and is rotated anteromedially to define its edge and associated inferior cornu. A needle-tipped electrocautery knife is used to separate the inferior constrictor from the thyroid lamina. The inferior cornu is identified and isolated so that the CT joint can be separated with a Mayo scissors. Separating the CT joint and the associated inferior constrictor muscle from the thyroid cartilage allows for further anteromedial rotation of the thyroid lamina.

The CT subluxation is accomplished by placing a 2-0 Prolene suture around the inferior cornu of the thyroid lamina (Fig 1A[10]). It is then passed in a submucosal fashion underneath the cricoid anteriorly. The suture is pulled taut (Fig 1B[10]) and increases the distance between the cricoarytenoid joint and the attachment of the anterior commissure ligament. This maneuver increases the tension and length of the musculomembranous vocal fold on the paralyzed side, thus closely simulating CT muscle contraction, so that pitch variation is easily controlled by the operator. The tension on this suture is adjusted by using a slipknot while the patient performs a number of phonatory gestures. These include maximal range tasks such as frequency variation (pulse register [vocal fry] through a falsetto register), glissando sliding scales, and maximal phonation time. The surgeon selects an adequate tension for affixing the suture by ensuring that the patient has an appropriate fundamental frequency (F0) with comfortable conversation-level effort, as well as the ability to obtain a falsetto register.

Vocal Function Measures. All 13 patients under-

TABLE 1. RESULTS OF ACOUSTIC ANALYSIS

		Avg F0 Reading (Hz)		*Avg SPL Reading (dB)*		*Max SPL Range (dB)*		*Max F0 Range (Hz)*		*Max Phonation Time (s)*		*Jitter (%)*		*Shimmer (%)*		*Noise-to-Harmonics Ratio*	
Subjects		*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
Male	1	169	159	62	64	*47*	*41*	121-430	*63-633*	4.5	*18.4*	6.58	2.34	11.93	15.27	0.204	*0.178*
	2	*	*143*	*70*	*74*	*41*	*51*	121-373	*86-544*	1.6	5.7	*	*0.39*	*	*3.78*	*	*0.116*
	3	157	*104*	67	*72*	*52*	*52*	85-204	*64-419*	13.3	*36.8*	3.00	*0.561*	14.13	*3.42*	0.209	*0.126*
	4	249	*135*	68	*72*	15	*72*	250-633	*86-503*	5.1	17.5	*	*0.29*	*	*1.51*	*	*0.134*
	5	151	*116*	67	69	*43*	*46*	*94-572*	*91-605*	12.9	*20.0*	*0.70*	*0.75*	*3.47*	*3.73*	*0.135*	*0.158*
Female	1	*232*	169	56	63	26	*43*	193-347	*132-566*	4.2	*16.9*	6.13	*0.85*	12.11	4.20	0.492	*0.113*
	2	*194*	*183*	66	67	31	37	160-553	*139-660*	8.9	*14.2*	2.51	*1.06*	6.71	4.03	*0.170*	*0.117*
	3	168	*198*	62	65	36	*47*	110-408	*129-689*	2.8	6.8	*1.33*	*1.30*	4.79	*3.71*	*0.151*	*0.140*
	4	172	173	65	65	35	*47*	*72-647*	*66-992*	10.9	*21.3*	*	1.41	*	4.54	*	*0.137*
	5	149	159	63	65	*45*	*60*	115-307	*133-543*	6.0	13.0	1.46	*1.38*	5.53	*2.26*	0.196	*0.113*

Italics indicate that value fell within normal limits.

Normal values used for comparisons — average F0 reading: males 82 to 145 Hz, females 180 to 240 Hz; average SPL reading: males ≥70 dB, females ≥68 dB; maximum SPL range ≥40 dB; maximum F0 range ≥2 octaves; maximum phonation time: males ≥18 s, females ≥14 s; jitter ≤1.4%; shimmer ≤3.81%; noise-to-harmonics ratio ≤0.19.

F0 — fundamental frequency, SPL — sound pressure level.

*Acoustic signal was too aperiodic to analyze.

went preoperative voice assessments. Postsurgical stroboscopic evaluations were available in all of the 13 patients, and complete postoperative objective measurements have been obtained thus far on 10 of the 13. The objective voice evaluation consisted of videolaryngoscopy with stroboscopy, as well as acoustic and aerodynamic measurements. The details of this protocol have been previously described.[9] The postoperative objective voice evaluations occurred within 4 months of the procedure. Long-term data are not yet available.

RESULTS

Thirteen patients underwent CT subluxation with other medialization procedure(s): 9 of the 13 had adduction arytenopexy and implant medialization laryngoplasty, and 4 of the 13 had medialization laryngoplasty alone. The medialization of the musculomembranous vocal fold was done with Gore-Tex in the majority of cases.[10-12] Most patients were discharged on the first postoperative day. The CT subluxation was complication-free and was typically done in under 10 minutes, since the exposure had already been obtained for the other procedures.

Acoustic Assessment. Results for acoustic assessment of oral reading (average F0 and sound pressure level [SPL]), maximum phonation tasks (F0 range, SPL range, and phonation time), and sustained vowels (jitter, shimmer, and noise-to-harmonics ratio [NHR]) are shown in Table 1 for the 10 patients who underwent both presurgical and postsurgical evaluation. Presurgically, 8 of the 10 patients displayed an abnormal average F0 for reading, and 9 had an abnormally low average SPL. Postsurgically, 8 patients showed improvement in average F0 (ie, migration of values toward the normal range), with 6 attaining normal values, while 9 displayed increases in average SPL, with 3 attaining a normal level.

For maximum phonation tasks, presurgical measures showed 5 of the 10 patients to have reduced maximum SPL ranges, 8 to have reduced maximum F0 ranges, and all 10 to have reduced maximum phonation times. Postsurgically, 8 of the 10 patients displayed increases in maximum SPL ranges, with all 10 showing increases in both maximum F0 ranges and maximum phonation times. Nine patients achieved normal postsurgical maximum SPL ranges, with all 10 demonstrating normal maximum F0 ranges, and 6 having normal maximum phonation times. Of the 4 patients who did not have normal maximum phonation times, 2 were within 1 second of a normal maximum phonation time, and the other 2 were 82 and 86 years old.

Presurgically, 3 of the 10 patients had voices that were too aperiodic to analyze, with 5 of the remaining 7 having abnormal jitter values, 6 displaying abnormal shimmer values, and 5 having abnormal NHR values. Postsurgically, all 10 patients displayed analyzable voice signals, with 8 having normal jitter values, 6 having normal shimmer values, and all 10 demonstrating normal NHR levels.

Aerodynamic Assessment. Results for aerodynamic testing are shown in Table 2. Presurgically, 4 of the 10 patients displayed abnormally high average airflow rates, and 7 had abnormally high subglottal pressure values. Postsurgically, all 10 patients had air-

TABLE 2. RESULTS OF AERODYNAMIC ANALYSIS

Subjects		*Average Flow (L/s)*		*Subglottal Pressure (cm H2O)*		*Sound Pressure Level (dB)*	
		Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*
Male	1	0.54	*0.15*	10.5	11.1	70.3	*78.2*
	2	0.98	*0.29*	21.8	8.9	84.2	82.8
	3	*0.18*	*0.11*	8.9	8.6	71.0	*77.8*
	4	1.64	*0.10*	16.2	11.6	*77.8*	79.8
	5	*0.28*	*0.18*	10.0	*7.3*	83.3	*76.5*
Female	1	*0.23*	*0.10*	*4.8*	*7.6*	63.2	69.0
	2	*0.10*	*0.15*	*6.9*	9.1	*78.0*	64.5
	3	0.40	*0.27*	*5.9*	9.4	62.8	70.5
	4	*0.11*	*0.18*	8.2	*6.5*	67.2	68.2
	5	*0.11*	*0.15*	12.3	*5.5*	60.7	71.8

Italics indicate that value fell within normal limits.

Normal values used for comparisons — average flow: males ≤0.32 L/s, females ≤0.28 L/s; subglottal pressure 3.3 to 8.1 cm H2O; sound pressure level 72.3 to 79.6 dB.

flow rates that fell within the normal range, with 6 showing a reduction in subglottal pressure, and 4 achieving subglottal pressures that were within the normal range for voice produced at a comfortable level.

Given the strong relationship between subglottal pressure and SPL, it is more meaningful to assess estimates of subglottal pressure relative to SPL.[13] Thus, presurgical and postsurgical subglottal pressure values for individual patients are plotted versus SPL in Fig 2.[13] Also shown for comparative purposes are the linear regression line and associated 90% confidence interval generated for data from a group of normal speakers.[13] Presurgically, the data values for all 10 patients fell above the normal confidence interval, indicating that higher-than-normal pressures were used by all patients to attain their measured SPL values. Postsurgically, data values migrated toward the normal interval for 5 patients, stayed at approximately the same distance from the normal interval for 2 patients (ie, changes between presurgical and postsurgical values ran essentially parallel to the normal interval), and moved further away from the normal interval for 3 patients. None of the postsurgical values decreased sufficiently to fall within the normal confidence interval.

Visual Assessment by Videolaryngoscopy With Stroboscopy. Preoperative examination during normal respiration revealed arytenoid corniculate hooding in 9 of the 13 patients; this was an indication of internal rocking and inferior displacement. All 13 patients demonstrated lateral arytenoid displacement in the horizontal plane, as well as bowing, and no epithelial lesions were noted.

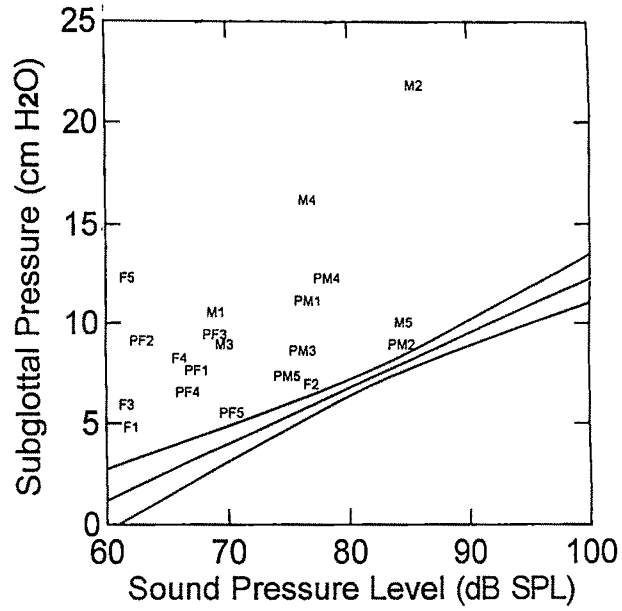

Fig 2. Subglottal air pressure measurements for individual patients versus sound pressure level for presurgical and postsurgical (indicated by "P" preceding patient's identification) assessments. Also shown is linear regression line and associated 90% confidence interval based on data from group of normal speakers.[13]

In 12 of the 13 subjects, a glottal gap extended from the intercartilaginous region into the musculomembranous region. Seven of the 13 had a very wide glottal chink (gap) encompassing most of the musculomembranous vocal fold, such that phonatory tasks resulted in an aperiodic flutter vibration with severe impairment of mucosal wave propagation.

Postoperatively, in all patients, 1) glottal closure was complete in the musculomembranous region, 2) hooding of the corniculate region was reduced, 3) the lateral displacement that was noted in the axial plane was improved dramatically, and 4) improvement was seen in the contour of the musculomembranous vocal fold, with almost complete resolution of bowing.

DISCUSSION

Cricothyroid subluxation was designed as an easily adjustable procedure to enhance the vocal outcome of other laryngoplastic phonosurgical procedures. It extends the normal vocal fold length and dramatically increases the viscoelastic tension of the denervated musculomembranous region. This is unlike all prior operations, which were designed primarily to treat paralytic dysphonia by repositioning the vocal fold edge.[6-9,14,15] Prior procedures that altered the tension and length of the vocal fold were conceived to modify pitch rather than to treat paralytic dysphonia.[8,16-18] The CT subluxation suture (Fig 1) simulates CT muscle contraction, produces countertension on the thyroarytenoid muscle, and increases the

TABLE 3. COMPARISON OF RESULTS BY PROCEDURE

Measures	*% Abnormal Presurgery*		*% Improved Postsurgery*		*% Normal Postsurgery*	
	AA + ML	*AA and/or ML + CT Sub*	*AA + ML*	*AA and/or ML + CT Sub*	*AA + ML*	*AA and/or ML + CT Sub*
Average Fo reading	67	80	44	80	56	60
Average SPL reading	78	90	78	90	56	30
Maximum SPL range	67	50	67	80	78	90
Maximum Fo range	78	80	33	100	22	100
Maximum phonation time	100	100	100	100	44	60
Jitter	89	80	89	100	67	80
Shimmer	100	90	89	90	22	60
Noise-to-harmonics ratio	100	70	100	80	67	100
Average flow	56	40	56	40	89	100
Subglottal pressure	89	70	78	60	0	40

Comparison is of results for patients who had adduction arytenopexy (AA) and medialization laryngoplasty (ML)[9] with results for patients who had new cricothyroid subluxation (CT Sub) procedure as well as ML, alone or combined with AA.

length of the musculomembranous vocal fold.[10] In the present study, CT subluxation was relatively easy to perform, was free from complications, and improved the acoustic outcome of the other laryngoplastic phonosurgical procedures.

A comparison of acoustic and aerodynamic results from an earlier study[9] of patients who had undergone adduction arytenopexy and medialization laryngoplasty with the results from the current study (new subluxation procedure, as well as medialization laryngoplasty alone or combined with adduction arytenopexy) is shown in Table 3.[9] These two studies displayed comparable percentages of patients who had abnormal presurgical measurement values. Postsurgically, there was a higher percentage of patients in the present study who displayed both postsurgical improvements and the attainment of normal measurement values, as compared to the group of patients who had undergone adduction arytenopexy and medialization laryngoplasty in the previous study.

Most noteworthy was the observation that all 10 patients in the present study displayed normal maximum F0 ranges, as compared to only 2 (22%) patients who attained normal (reliable) F0 ranges in the previous study. (Note: patient performance of maximum F0 range tasks was so variable and unreliable that the actual data values were not reported in the previous study.) Additionally, all patients approached a normal maximal phonation time, except the 2 oldest individuals. They did improve substantially, and we suspect that normative data for individuals over 80 would reveal figures closer to these subjects' results.

It is not clear to what extent high postsurgical subglottal pressures are due simply to the persistence of presurgical hyperfunctional behaviors, or if the surgically altered laryngeal mechanism requires higher pressures to produce as normal an acoustic output as possible. We are currently in the process of trying to determine this by giving postsurgical patients voice therapy in an attempt to reduce the driving pressures while maintaining voice quality and flexibility.

The adduction arytenopexy resulted in improvements in a number of objective measures of vocal function. This procedure simulates normal glottal closure by reproducing the synchronous adductor contractile characteristics of the lateral cricoarytenoid, interarytenoid, lateral thyroarytenoid, and posterior cricoarytenoid muscles. However, vocal outcome measures revealed limitations in maximal range tasks, especially dynamic frequency range (Table 3). This finding was most likely due to persistent flaccidity of the denervated thyroarytenoid musculature. Both Isshiki's[7,8] and Zeitels'[9] arytenoid adducting techniques separate the CT joint to expose the cricoarytenoid joint. During a collaborative cadaver dissection, R. Sataloff (personal communication, 1997) observed that separation and destabilization of the ipsilateral CT joint impairs the function of the contralateral CT muscle. On the basis of a similar observation, other authors have exposed the cricoarytenoid joint during arytenoid adduction by removing a posterior window of the thyroid lamina and leaving the CT joint intact.[19]

Clinical observations revealed that after the CT joint was opened, the inferior cornu of the thyroid lamina became retrodisplaced with relation to the cricoid. This meant that the previously fixed vocal fold length was shortened, and the tension of the thyroarytenoid muscle was reduced. Essentially, there was a decrease in the normal distance between the cricoarytenoid joint and the insertion of the anterior commissure tendon into the thyroid lamina.

We believe that the foreshortening of the paralyzed vocal fold, induced by separating the CT joint, led to compensatory hyperfunctional foreshortening of the normal vocal fold. This hyperfunctional adaptation occurs to align the vocal processes — an alignment that is a prerequisite for normal entrained oscillation during phonation. The decreased phonatory length of both vocal folds and the decreased viscoelastic tension of the paralyzed vocal fold resulted in limitations in acoustic maximal range capabilities (Table 3). Despite the fact that the paralyzed vocal fold was surgically placed under higher tension (than its preoperative state) after CT subluxation, almost all patients could reach a lower frequency (Table 1). This paradox is probably explained by the fact that the postsubluxation-lengthened vocal fold reduces contralateral vocal fold hyperfunction.

The CT subluxation procedure was designed to rectify these mechanical impediments, which were partially precipitated by disruption of the CT joint during laryngoplastic phonosurgical correction of paralytic dysphonia. The CT subluxation also improved the vocal outcome of those patients who did not require an arytenoid procedure (because of somewhat favorable synkinesis). In both scenarios, the objective measures reflect that CT subluxation improved the aerodynamic efficiency of the glottal valve, with a commensurate enhancement of the maximal range acoustic characteristics of the voice (Table 3). Subjective perceptions revealed that most patients demonstrated a register transition between modal and falsetto, an observation not previously reported. We are currently developing measurement approaches to demonstrate this *passagio*.

CONCLUSIONS

The CT subluxation procedure is an easily adjustable method of increasing the tension and length of the paralyzed vocal fold. It is the first vocal fold paralysis procedure that is primarily designed for this purpose rather than to reposition the vocal fold edge. The suture simulates contraction of the CT muscle. In the present study, CT subluxation was easy to perform, was unassociated with complications, and enhanced laryngoplastic procedures that medialize the arytenoid and/or musculomembranous vocal fold. Objective measures of vocal function revealed that all patients achieved normal dynamic pitch variation, glottal airflow, and NHR. With the addition of the CT subluxation procedure to the armamentarium of the phonosurgeon, all parameters for static reconstruction of the paralyzed vocal fold have been addressed. Long-term analysis (>6 months) is necessary to confirm the efficacy of these encouraging results. This caveat notwithstanding, future developments for treatment of paralytic dysphonia should lie primarily in dynamic reconstruction (reinnervation and electrical pacing) of the intrinsic laryngeal musculature.

REFERENCES

1. Payr E. Plastik am schildknorpel zur Behebung der Folgen einseitiger Stimmbandlahmung. Dtsch Med Wochenschr 1915;43:1265-70.

2. Meurman Y. Mediofixation der Stimmlippe bei ihrer vollstandigen Lahmung. Arch Ohren Nasen Kehlkopfheilkd 1944; 154:296-302.

3. Meurman Y. Operative mediofixation of the vocal cord in complete unilateral paralysis. Arch Otolaryngol 1952;55:544-53.

4. von Leden H. The history of phonosurgery. In: Ford CN, Bless DM, eds. Phonosurgery assessment and surgical management of voice disorders. New York, NY: Raven Press, 1991:3-24.

5. Sawashima M, Totsuka G, Kobayashi T. Reconstructive surgery for hoarseness due to unilateral vocal cord paralysis. Arch Otolaryngol 1968;87:226-9.

6. Isshiki N, Morita H, Okamura H, Hiramoto M. Thyroplasty as a new phonosurgical technique. Acta Otolaryngol (Stockh) 1974;78:451-7.

7. Isshiki N, Tanabe M, Sawada M. Arytenoid adduction for unilateral vocal cord paralysis. Arch Otolaryngol 1978;104: 555-8.

8. Isshiki N. Phonosurgery: theory and practice. Tokyo, Japan: Springer-Verlag, 1989.

9. Zeitels SM, Hochman I, Hillman RE. Adduction arytenopexy: a new procedure for paralytic dysphonia with implications for implant medialization. Ann Otol Rhinol Laryngol Suppl 1998;107(suppl 173).

10. Zeitels SM. Adduction arytenopexy with medialization laryngoplasty and crico-thyroid subluxation: a new approach to paralytic dysphonia. Operative Techniques Otolaryngol Head Neck Surg 1999;10:9-16.

11. Hoffman HH, McCulloch TM. Medialization laryngoplasty with Gore-Tex. Operative Techniques Otolaryngol Head Neck Surg 1999;10:6-8.

12. McCulloch TM, Hoffman HT. Medialization laryngoplasty with expanded polytetrafluoroethylene. Surgical technique and preliminary results. Ann Otol Rhinol Laryngol 1998; 107:427-32.

13. Holmberg EB, Hillman RE, Perkell JS, Gress C. Relationships between intra-speaker variation in aerodynamic measures of voice production and variation in SPL across repeated recordings. J Speech Hear Res 1994;37:484-95.

14. Koufman JA. Laryngoplasty for vocal cord medialization: an alternative to Teflon. Laryngoscope 1986;96:726-31.

15. Koufman JA, Isaacson GL. Laryngoplastic phonosurgery. Otolaryngol Clin North Am 1991;24:1151-77.

16. Isshiki N. Recent advances in phonosurgery. Folia Phoniatr 1980;32:119-54.

17. LeJeune FE, Guice CE, Samuels PM. Early experiences with vocal ligament tightening. Ann Otol Rhinol Laryngol 1983; 92:475-7.

18. Tucker HM. Anterior commissure laryngoplasty for adjustment of vocal fold tension. Ann Otol Rhinol Laryngol 1985; 94:547-9.

19. Miller FR, Bryant GL, Netterville JL. Arytenoid adduction in vocal fold paralysis. Operative Techniques Otolaryngol Head Neck Surg 1999;10:36-41.

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MOTOR INNERVATION OF THE HUMAN CRICOPHARYNGEUS MUSCLE

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Innervation of the human cricopharyngeus muscle remains historically controversial and unclear, encouraging numerous treatments inconsistently designed to pharmacologically or mechanically alter the contractile state of this muscle. Neuroanatomic controversy results from and is perpetuated by 1) use of nonhuman models, 2) observational misinterpretation of small-diameter, overlapping nerve fibers, and, most importantly, 3) lack of real-time verification of neural projections. We sought to overcome these difficulties by performing microdissections in 27 patients undergoing laryngectomy and using real-time electromyographic verification. We demonstrated 1) dual ipsilateral innervation by the pharyngeal plexus and recurrent laryngeal nerve, 2) segmental projection of the recurrent laryngeal nerve to anterior motor units, 3) pharyngeal plexus projection to posterior motor units, 4) absence of a sympathetic or external superior laryngeal nerve contribution, and 5) absence of contralateral innervation. Such dual ipsilateral innervation, segmentally projected, has not been previously described in any other form of neuromuscular organization. Neuroanatomic accuracy should improve diagnostic and therapeutic strategies for future management of pharyngeal dysphagia.

KEY WORDS — cricopharyngeus muscle, electromyography, motor innervation.

INTRODUCTION

Of all the neuromuscular units essential to normal swallowing, none surpass the human cricopharyngeus muscle (CPM) in capturing our scientific curiosity and clinical imagination. The innervation of this muscle unit remains especially mysterious, often misinterpreted and controversial. Lack of clarity has already encouraged numerous treatments, inconsistently intended to pharmacologically or mechanically alter its contractile state. Understandably, clinical benefits have remained inconsistent at best.

As an anatomic entity, the CPM represents a dorsal midline structure, bilaterally arising from the lateral laminae of the cricoid cartilage, and, with the posterior cricoid lamina, encircling the entrance of the esophagus (Fig 1). Physiologically, its baseline tonic contraction gives way to a brief inhibitory state preceding the passage of a swallowed bolus, followed by a burst of contraction during the height of swallow.[1-4] It notably exhibits phasic contraction during the inspiratory portion of the respiratory cycle in the dog,[5] and contraction during phonatory effort in the cat and dog.[5,6]

A focused review of the literature shows that neuroanatomic controversy results from and is perpetuated by the following historical choices: 1) use of nonhuman models and 2) observational misinterpretation of small-diameter, overlapping nerve fibers, caused in part by 3) lack of real-time validation of motor projections.

In defining motor control of the CPM, most investigators implicate an innervating network of small-diameter fibers constituting what is termed the pharyngeal plexus (PP) of the vagus nerve. Such innervation was first described in part in the dog by Lemere[7] in 1932 and confirmed by Lund and Ardran[8] in 1964. Sprague[9] described this relationship in monkeys and humans in 1944, while Mu and Sanders[10] elegantly reconfirmed it in humans in 1996.

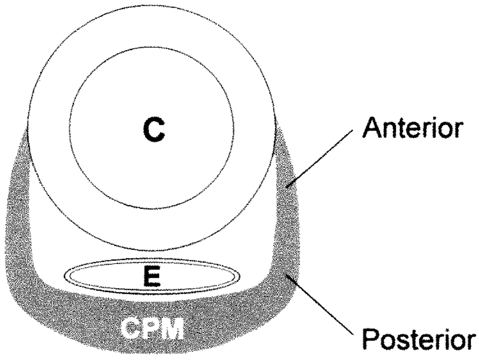

Fig 1. Schematic drawing demonstrating axial relationship of cricoid cartilage (C) to cricopharyngeus muscle (CPM) and esophagus (E). Anterior and posterior portions of CPM are indicated.

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Alternatively, Hollinshead[11] in 1954 suggested the CPM was innervated by terminal branches of the recurrent laryngeal nerve (RLN). This observation was in part further supported in 1948 by Hwang et al[12] in the monkey, rat, and guinea pig, and was supported in humans by Rustard and Morrison,[13] Steinberg et al,[14] and Mu and Sanders.[10]

Further, there were a number of thoughtful observors who suggested the CPM was also innervated by the terminal external division of the superior laryngeal nerve (eSLN). Sprague[9] described it in the monkey and dog, Hwang et al[12] in the monkey, rat, and guinea pig, and Hollinshead[11] in humans.

More controversially, a group of coworkers from our laboratory had implicated possible sympathetic innervation, suggesting that CPM function was at least under partial autonomic motor control. As early as 1939, Sjoberg[15] suggested that sympathetic stimulation produced CPM contraction, whereas cholinergic stimulation brought CPM relaxation in cats. A rationale for this position was supported 4 years earlier, when Rogers[16] reported relief of dysphagia in a woman on whom he performed bilateral cervical sympathectomies. The role of cervical sympathetic innervation was further strengthened by Kirchner's[17] canine model that received the Triological Society's Mosher Award in 1958 and was again confirmed by Murakami et al[18] in the cat model in 1972.

To add to the controversy, Lund and Ardran,[8] as well as Murakami and Kirchner,[6] rejected RLN innervation in the dog and cat, respectively, while Hollinshead[11] rejected a sympathetic supply in humans. Others then questioned and rejected the possibility of crossed innervation.[19]

Thus, from a focused review of the literature, the critical student will find the CPM curious, challenging, and mysterious. Is it possible that the CPM may not be innervated by a single nerve, but by several? Is it conceivable that it could be cross-innervated or that at least part of its function is governed by autonomic motor control? The succeeding effort is intended to clarify the motor innervation of the human CPM, enhance our appreciation of its form and diverse functions, and, hopefully, reduce the mystery of its role in clinical laryngology.

MATERIALS AND METHODS

Again, a review of the literature shows that several sources of confusion may have contributed to neuroanatomic controversy: 1) use of nonhuman models, 2) observational misinterpretation of small-diameter, overlapping nerve fibers, and 3) lack of real-time validation of motor projections.

We sought to overcome these difficulties by performing microdissections in a total of 27 patients (22 male, 5 female) undergoing total laryngectomy for T3 or T4 endolaryngeal cancer and by using real-time electromyography (EMG) verification of motor nerve projections. Patients were maintained at 1 MAC (minimal alveolar concentration) isoflurane anesthesia without chemical muscle relaxation. The core body temperature was carefully stabilized at 38°C with a warming blanket. In accordance with institutional review requirements, this project was approved by the Yale Human Investigation Committee (protocol #10089).

Protocol 1. CPM Innervation With EMG Validation. In the first subgroup of 9 patients (7 male, 2 female), careful microdissections of the PP, RLN, eSLN, and sympathetic plexus were accomplished under microscopic guidance. In order to verify their projections, a monopolar Teflon-coated 26-gauge stainless steel electrode was used to stimulate each isolated nerve while EMG recordings of the CPM were accomplished bilaterally with bipolar platinum iridium electrodes (0.3 mm diameter, 25 kΩ). An XLTEK Neuromax provided the capability of neural stimulation while recording compound M-wave potentials on-line and in real time, M-waves representing muscle potentials produced by directly stimulating the muscle's motor nerve. This method also provided storage capability for later off-line data analyses. Stimulation parameters consisted of single-shock rectangular waves of 0.5 to 4.0 mA and 0.1-millisecond pulse duration, with maximal stimulus amplitudes restricted in this protocol to less than 1.0 mA when possible.

Protocol 2. Effect of Sequential Denervation on Tonic CPM Activity. In an additional subgroup of 9 patients (8 male, 1 female), the effect of sequential CPM denervation on tonic spontaneous activity was ascertained. Resting EMG provided an opportunity to compare spike densities measured immediately before and after PP section followed by RLN section. These observations were accomplished to confirm the specific neural bases of CPM innervation identified in protocol 1.

Protocol 3. Mapping of Neural Projections to CPM. In a subsequent subgroup of 6 patients (5 male, 1 female), the relative topographic composition of RLN/PP motor unit ratios was assessed by comparing M-wave amplitudes at threshold from 2 separate locations on the CPM: anterior and posterior (Fig 1). The relative peak-to-peak amplitude of compound muscle action potentials (CMAPs) derived when the PP and RLN were stimulated *sequentially* compared

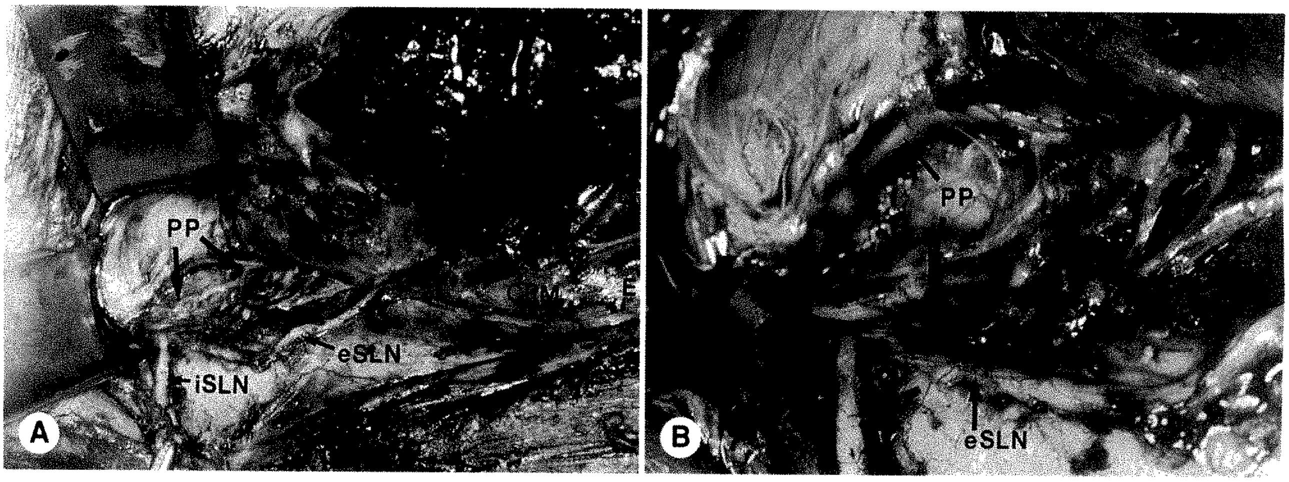

Fig 2. Operative dissection of right side of patient's neck. A) Unmagnified view demonstrates relationship of external division of superior laryngeal nerve (eSLN) and internal branch of superior laryngeal nerve (iSLN) to pharyngeal plexus (PP). Note positions of cricopharyngeus muscle (CPM), esophagus (E), and trachea (T). B) Magnified view demonstrates that eSLN and PP are distinct and separate nerves.

to CMAP amplitudes derived when they were stimulated *simultaneously* provided a means for topographic assessment of relative RLN/PP contributions in each muscle location. That is to say, the ratio of CMAP amplitude produced by RLN stimulation to the sum of amplitudes produced by sequential RLN and PP stimulation provided an estimation characterized by the ratio (expressed in percent) of RLN-innervated muscle fibers to total muscle fibers in any specified location of the CPM. Conversely, the ratio of CMAP amplitude produced by PP stimulation to the sum of amplitudes produced by sequential RLN and PP stimulation likewise provided an estimation characterized by the ratio or percentage of PP-innervated muscle fibers to total muscle fibers in any given location of the CPM. Mathematically expressed, $[A/(A + B)] \times 100 = \%A$, and $[B/(A + B)] \times 100 = \%B$. This rationale was based on the assumption that the amplitude of the CMAP reflects the number of muscle fibers activated by nerve stimulation and represents the sum of amplitudes of those individual muscle fibers.[20]

Protocol 4. Histochemical Mapping of CPM Fiber Types (Myofibrillar ATPase). In a final subgroup of 3 patients (2 male, 1 female), muscle histochemistry was performed to determine the relative compositions of type I and type II fibers in the anterior compartment of the CPM as compared to its posterior compartment for correlation against the topographic EMG analyses obtained in protocol 3. The CPM samples from each topographic location were sectioned at 10 µm, post fixed in methanol-free formalin, and immersed in 0.1-mol/L Tris–hydrochloric acid buffer containing 0.018-mol/L calcium chloride, pH 10.2. After rinsing, sections were then immersed sequentially in 2% cobalt nitrate and 0.5% ammonium sulfide. After counterstaining in methyl

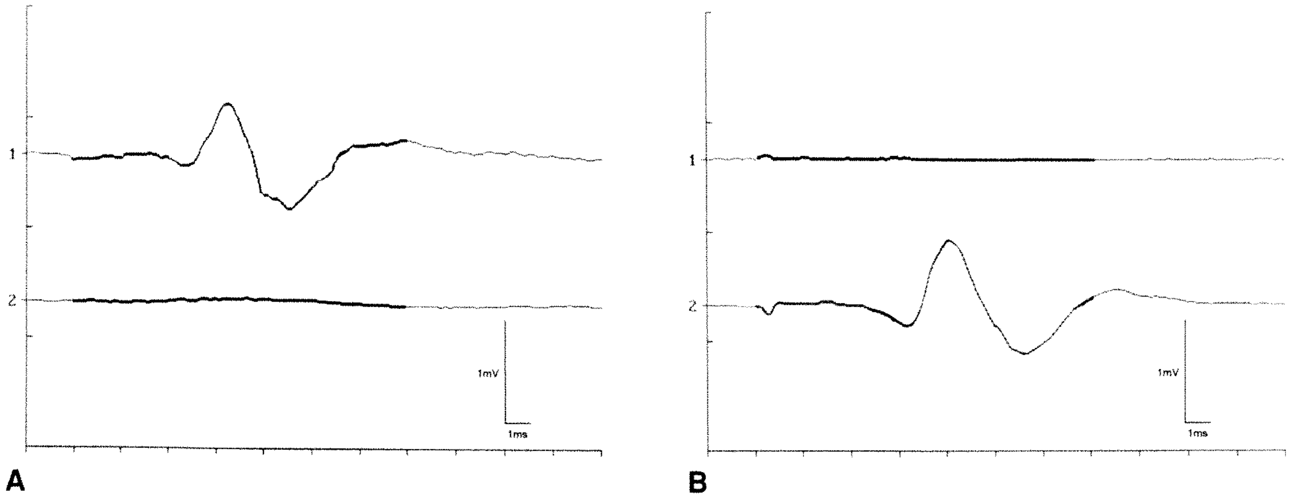

Fig 3. Compound muscle action potential (CMAP) of CPM produced by stimulation of A) right PP and B) left PP. 1 — Right CPM; 2 — left CPM. Note large-amplitude CMAP in ipsilateral CPM when either right or left PP is maximally stimulated.

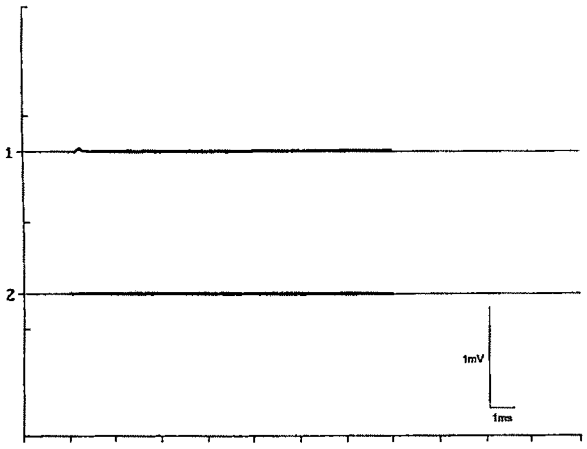

Fig 4. Maximal stimulation of right eSLN produces no M-wave in either right (1) or left (2) CPM.

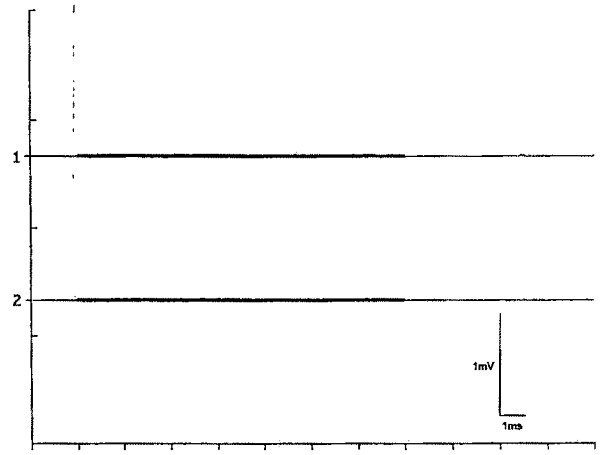

Fig 5. Maximal stimulation of right sympathetic nerve produces no M-wave in either right (1) or left (2) CPM.

green, sections were mounted and examined. The number of pale type I fibers and the number of dark type II fibers per high-powered field were averaged from 8 nonoverlapping microscopic fields per muscle sample.

RESULTS

Protocol 1. CPM Innervation With EMG Validation. Due to relatively large fiber diameters, identification of the RLN and cervical sympathetic fibers was not as technically demanding as the identification of the PP and eSLN. In contrast, the spatial relationship of the PP to the eSLN in the 9 subjects required more meticulous microdissection under 6× magnification, due, in part, to much smaller fiber size. In our experience, positive identification of each nerve could not have been accurately accomplished without real-time EMG validation, since their spatial relationships involved 3-dimensional overlap above the level of C2. Our dissections distinctly demonstrated the eSLN and PP as separate entities despite their overlapping proximity to each other (Fig 2). Single-shock maximal stimulation of the PP at 1.0 mA reliably produced a large-amplitude 1.0-mV CMAP in the mid-portion of the ipsilateral CPM (Fig 3). On the other hand, no M-wave was observed with up to 4.0 mA stimulation of the eSLN or sympathetic plexus (Figs 4 and 5). Maximal stimulation of the RLN produced lower-amplitude 0.5-mV CMAPs in the mid-portion of the ipsilateral CPM (Fig 6). (Due to the proximity of the surgically exposed RLN to the CPM, often less than 5 mm apart, control stimulation of adjacent non-neural paratracheal tissue was performed and showed no measurable CPM M-wave potential, thus eliminating the possibility of direct current spread to the CPM during RLN stimulation.) It is also important to note that no contralateral CMAP was produced with maximal or supramaximal stimulation of any nerve, which thus also eliminated the suggestion that the CPM may be cross-innervated.

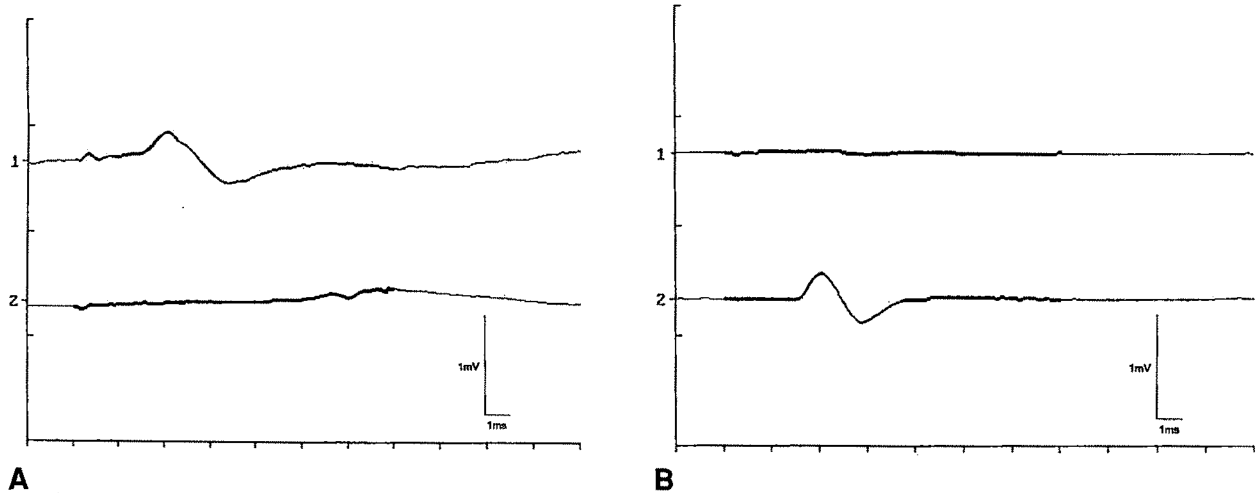

Fig 6. CMAP of CPM produced by stimulation of A) right recurrent laryngeal nerve (RLN) and B) left RLN. 1 — Right CPM; 2 — left CPM. Note lower-amplitude CMAP in ipsilateral CPM when either right or left RLN is maximally stimulated.

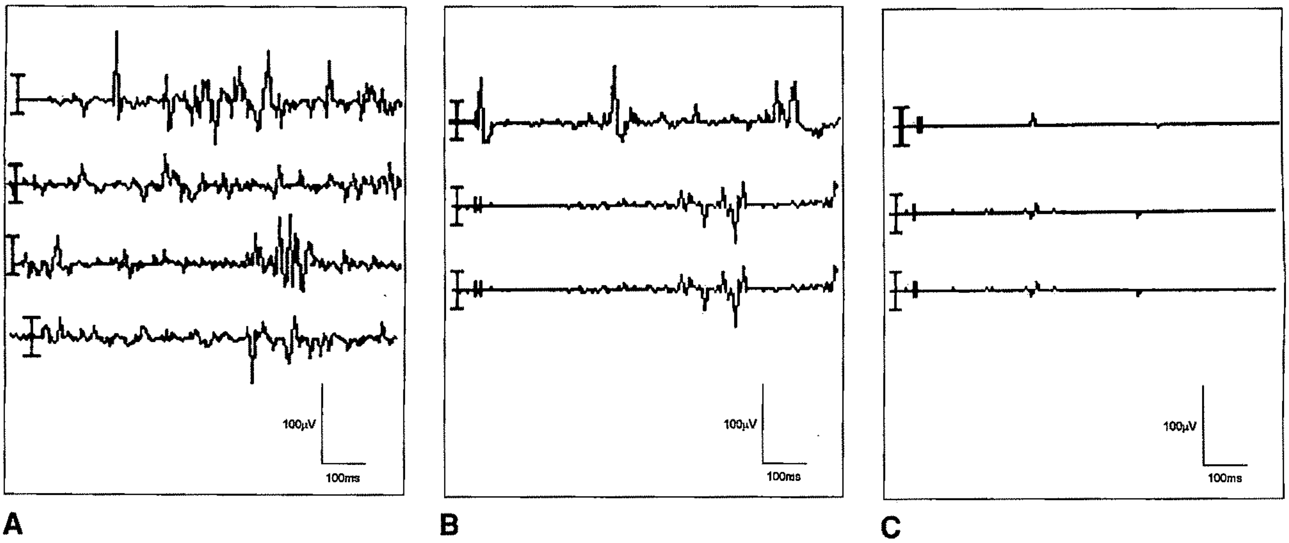

Fig 7. Spontaneous CPM spike activity seen A) at baseline, B) after pharyngeal nerve is sectioned, and C) after both pharyngeal nerve and RLN are sectioned.

Rather, these data suggest *dual ipsilateral* innervation of the CPM by both the PP *and* the RLN.

Protocol 2. Effect of Sequential Denervation on Tonic CPM Activity. With recording electrodes positioned at the mid-portion of the CPM (Fig 7), the tonic spike density was calculated at baseline and compared to spike counts resulting after section of the PP. All spikes with amplitudes over 50 µV were counted and averaged over 4 sample intervals or epochs, each sample width measuring 1.0 second. In a typical patient, the average CPM spike count was 10.25 before nerve section, and the average spike count was 7.33 after ipsilateral PP section (p = .270; Table 1). When the ipsilateral RLN was additionally sectioned, the average spike count in a typical patient was 0.0; when it was compared to the average spike count of 7.33 obtained after PP section alone, the resulting p value was .001. Similar findings were observed in all 9 subjects. Therefore, in any given patient after PP section alone, the spike density decreased, but did not produce a statistically significant difference from the control. Statistical significance was only achieved after additional ipsilateral RLN section was accomplished, confirming *dual ipsilateral* innervation by both the PP *and* the RLN.

TABLE 1. EFFECT OF SEQUENTIAL DENERVATION

		Spikes per Second
Spontaneous activity[a]		21
		4
		13
		3
	Average	10.25
PP section[b]		8
		7
		7
	Average	7.33
RLN section[c]		0
		0
		0
	Average	0

a versus b, p = .2703.
b versus c, p = .0010.
PP — pharyngeal plexus, RLN — recurrent laryngeal nerve.

Protocol 3. Topographic Mapping of Neural Projections to CPM. In 2 initial subjects, the peak-to-peak CMAP amplitude obtained at threshold from *simultaneous* RLN and PP stimulation appeared to correlate with the sum of amplitudes obtained when PP and RLN stimulation occurred *sequentially* (Table 2). This observation formulated the basis for an assumption that the ratio of CMAP amplitude produced by RLN stimulation to the sum of amplitudes produced by sequential RLN and PP stimulation provided an estimation characterized by the ratio (expressed in percent) of RLN-innervated muscle fibers to total innervated muscle fibers in any specified location of the CPM. Conversely, the ratio of CMAP amplitude produced by PP stimulation to the sum of

TABLE 2. EFFECT OF SEQUENTIAL RLN AND PP STIMULATION COMPARED TO SIMULTANEOUS RLN AND PP STIMULATION

	CMAP (mV)	
	Patient A	*Patient B*
RLN stimulation alone	152.6	104.9
PP stimulation alone	2,222.9	2,033.7
Sum	2,375.5	2,138.6
Simultaneous RLN and PP stimulation	2,324.2	2,142.3

CMAP — compound muscle action potential.

TABLE 3. TOPOGRAPHIC MAPPING OF NEURAL PROJECTIONS

Patient No.	*Sex*	*Age*	*Anterior CPM*		*Posterior CPM*	
			RLN Stimulation	*PP Stimulation*	*RLN Stimulation*	*PP Stimulation*
1	F	65	0.163 (89.31%)	0.020 (10.69%)	0.043 (10.64%)	0.361 (89.36%)
2	M	52	0.222 (65.49%)	0.103 (34.51%)	0.164 (8.06%)	1.870 (91.94%)
3	M	66	0.255 (13.99%)	1.567 (86.01%)	0.101 (4.52%)	2.133 (95.48%)
4	M	61	0.117 (28.19%)	0.404 (71.81%)	0.031 (4.34%)	0.683 (95.66%)
5	M	51	0.066 (13.41%)	0.426 (86.59%)	0.054 (12.82%)	0.459 (87.18%)
6	M	45	0.149 (32.39%)	0.311 (67.61%)	0.092 (20.28%)	0.495 (79.72%)

Data are millivolts.

CPM — cricopharyngeus muscle.

amplitudes produced by sequential RLN and PP stimulation likewise provided an estimation characterized by the ratio or percentage of PP-innervated muscle fibers to total innervated muscle fibers in any given location of the CPM. Mathematically expressed, $[A/(A + B)] \times 100 = \%A$, and $[B/(A + B)] \times 100 = \%B$. Therefore, in 6 additional subjects, topographic mapping of neural projections in the anterior CPM could then be compared to mapping of those in the posterior CPM (Table 3).

Two distinct subpopulations of subjects emerged. One group (patients 1 and 2) exhibited predominantly RLN muscle fibers anteriorly, whereas the other group (patients 3 through 6) exhibited predominantly PP fibers anteriorly. However, in either case, the percentage of RLN-innervated muscle fibers *decreased* from anterior to posterior, whereas the percentage of PP-innervated muscle fibers *increased* from anterior to posterior.

Protocol 4. Histochemical Mapping of CPM Fiber Types. In 4 sample sides, obtained from 3 patients, a comparison of type I and type II CPM fiber composition was evaluated anteriorly and posteriorly by muscle histochemistry (Fig 8). No clear mosaic pattern emerged to correlate fiber composition with the topographic patterns suggested by EMG, in that anteriorly, type II fibers predominated in 3 of 4 sides, and posteriorly, type II predominated in all 4 sides (Table 4).

DISCUSSION

These data uniformly and exclusively demonstrate dual ipsilateral CPM innervation by both the PP and the RLN. However, topographic electrophysiological analysis distinguishes 2 separate mosaic patterns of neural projections. That is to say, in the first group, RLN-innervated muscle fibers predominate anteriorly, whereas in the larger, second group, PP-innervated muscle fibers seem to predominate anteriorly. However, in both groups, the percentage of RLN muscle fibers *decreases* from anterior to posterior, while PP muscle fibers *increase* from anterior to posterior. Such topographic distribution of motor units, not surprisingly, correlates with the respective entry sites of the RLN (anteriorly) and the PP (posteriorly) into the CPM (Fig 9). That intramuscular neural arborization is greater proximally than distally is again consistent with this observation. Although there was a poor correlation seen by histochemistry that may be related to sample size, the heterogeneous composition of muscle fiber types, respectively innervated by the RLN and PP, may truly reflect a generalized neuromuscular heterogeneity characteristic of all pharyngeal muscles.

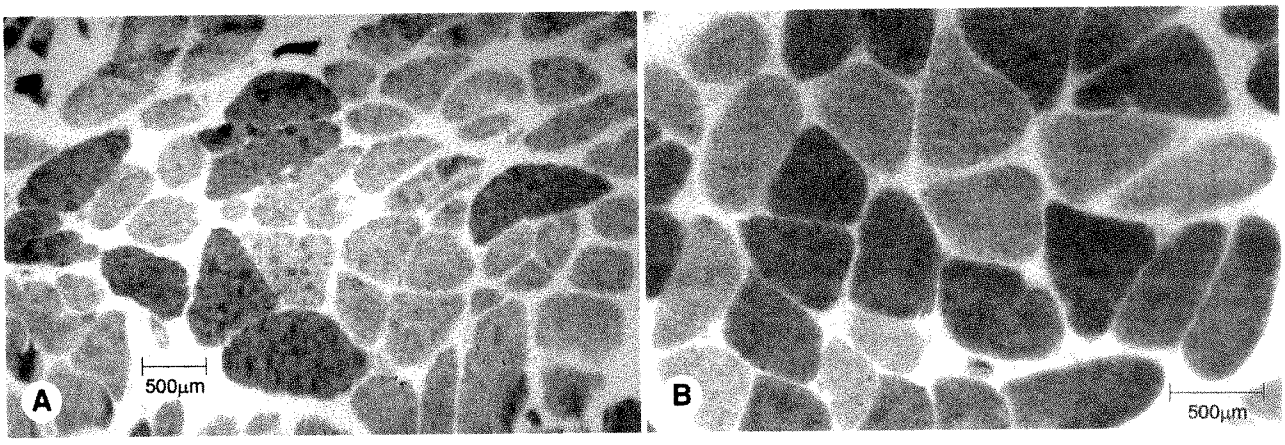

Fig 8. Typical ATPase histochemical profile of A) anterior and B) posterior portions of CPM. No obvious gross differences are noted.

TABLE 4. COMPOSITION OF CPM ACCORDING TO TYPE I AND TYPE II FIBERS

		Anterior CPM		*Posterior CPM*	
Side		*Type I Fibers*	*Type II Fibers*	*Type I Fibers*	*Type II Fibers*
1		10 (21.7%)	36 (78.3%)	13 (28.3%)	33 (71.7%)
		4 (13.8%)	25 (86.2%)	13 (22.8%)	44 (77.2%)
		7 (17.5%)	33 (82.5%)	41 (49.4%)	42 (50.6%)
		17 (26.6%)	47 (73.4%)	39 (45.9%)	46 (54.1%)
		15 (26.3%)	42 (73.7%)	32 (41.6%)	45 (58.4%)
		18 (21.7%)	65 (78.3%)	20 (35.7%)	36 (64.3%)
		15 (17.2%)	72 (82.8%)	37 (46.8%)	42 (53.2%)
		11 (14.1%)	67 (85.9%)	24 (40.0%)	36 (60.0%)
	Average	19.9%	80.1%	38.8%	61.2%
2		15 (51.7%)	14 (48.3%)	26 (40.6%)	38 (59.4%)
		33 (68.8%)	15 (31.3%)	20 (28.2%)	51 (71.6%)
		52 (82.5%)	11 (17.5%)	14 (26.4%)	39 (73.6%)
		51 (83.6%)	10 (16.4%)	23 (35.4%)	42 (64.6%)
		21 (56.8%)	16 (43.2%)	17 (31.5%)	37 (68.5%)
		32 (74.4%)	11 (25.6%)	16 (23.2%)	53 (76.8%)
		49 (87.5%)	7 (12.5%)	12 (27.3%)	32 (72.7%)
		31 (72.1%)	12 (27.9%)	10 (19.2%)	42 (80.8%)
	Average	74.7%	25.3%	29.2%	70.8%
3		60 (54.1%)	51 (45.9%)	31 (46.3%)	36 (53.7%)
		61 (49.2%)	63 (50.8%)	14 (38.9%)	22 (61.1%)
		62 (53.4%)	54 (46.6%)		
		43 (41.7%)	60 (58.3%)		
	Average	49.8%	50.2%	43.7%	56.3%
4		25 (41.0%)	36 (59.0%)	13 (22.0%)	46 (78.0%)
		26 (38.2%)	42 (61.8%)	12 (23.1%)	40 (76.9%)
		30 (49.2%)	31 (50.8%)	37 (52.9%)	33 (47.1%)
		26 (40.0%)	39 (60.0%)	32 (44.4%)	40 (55.6%)
		25 (43.9%)	32 (56.1%)		
		24 (49.0%)	25 (51.0%)		
		23 (43.4%)	30 (56.6%)		
		21 (42.0%)	29 (58.0%)		
	Average	43.3%	56.7%	37.2%	62.8%

Data are counts per high-powered field.

In addition to our observations that support ipsilateral PP and RLN innervation, the data express the notion that the CPM is likely derived embryologically from 2 separate anlagen of the vagal system: one associated with pharyngeal (PP) origin and the other associated with laryngeal (RLN) development. Phasic contraction of the CPM during the inspiratory portion of the respiratory cycle or during phonatory effort likely reflects its segmental laryngeal origins, while contractions of the CPM during the pharyngeal phase of swallow express its predominant pharyngeal origin. Just as its contraction during inspiration ensures against esophageal air entry as intrathoracic pressure approaches subatmospheric, so does its contraction during phonation theoretically stabilize vocal tract volume by ensuring against esophageal penetration. Our observations regarding the dual embryological origins of the CPM may now have the capability of supporting and extending our understanding of its diverse roles in all of those functions related to swallowing, breathing, and vocalizing.

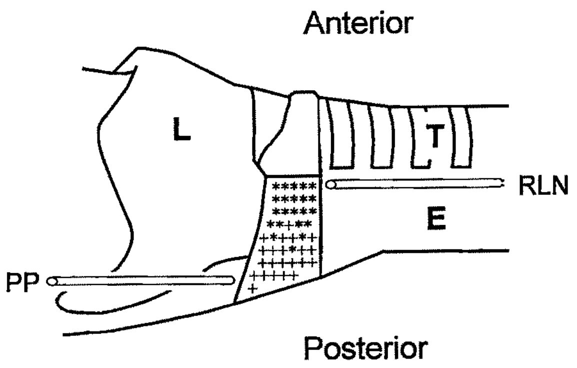

Fig 9. Lateral schematic drawing of pharynx and larynx demonstrating relative anterior CPM entry of RLN compared to posterior CPM entry of PP. Note positions of larynx (L), trachea (T), and esophagus (E).

Further, both excitation and denervation protocols clearly show an ipsilateral pattern of motor innervation, definitively supporting the concept that the CPM, as a midline structure, constitutes the embryological merger or fusion of 2 anatomic halves. There is no bilateral innervation of each half, a point of view supported functionally as well.[19]

Last, our data also contribute important exclusionary observations only made possible by real-time EMG validation. The data clearly fail to support CPM innervation by either the eSLN or cervical sympathetics. The notion that eSLN injury or neurectomy affects the contractile state of the CPM appears unlikely. Further, the suggestion that clinical treatment of the CPM can be accomplished pharmacologically by either adrenergic or cholinergic manipulation now appears remote.

In summary, the dual ipsilateral innervation of the CPM, segmentally projected, has not been previously described in any other form of neuromuscular organization. Neuroanatomic implications concerning the diverse embryological origins of the CPM are by themselves fascinating, as are consequent physiological implications for its separate deglutitory, respiratory, and phonatory functions. Above all, any incremental advance toward neuroanatomic accuracy can only improve future diagnostic and therapeutic strategies in the management of pharyngeal disease, while the orderly process of gathering those basic data can also be most gratifying.

REFERENCES

1. Shipp T, Deatsch WW. Pharyngoesophageal muscle activity during swallowing in man. Laryngoscope 1970;80:1-16.
2. Asoh R, Goyal KR. Manometry and electromyography of the upper esophageal sphincter in the opossum. Gastroenterology 1978;74:514-20.
3. van Overbeek JJM, Wit HP, Paping HL, Segenhout HM. Simultaneous manometry and electromyography in the pharyngoesophageal segment. Laryngoscope 1985;95:582-4.
4. Ertekin C, Pehlivan M, Aydogdu I, et al. An electrophysiological investigation of deglutition in man. Muscle Nerve 1995;18:1177-86.
5. Kawasaki M, Ogura J, Takenouchi S. Neurophysiologic observations of normal deglutition. I. Its relationship to the respiratory cycle. Laryngoscope 1964;74:1747-65.
6. Murakami Y, Kirchner JA. Respiratory activity of the external laryngeal muscles: an electromyographic study in cat. In: Wyke B, ed. Ventilatory and phonatory control systems. London, England: Oxford University Press, 1974:430-58.
7. Lemere F. Innervation of the larynx. Am J Anat 1932;51:417-37.
8. Lund WS, Ardran GM. The motor nerve supply of the cricopharyngeal sphincter. Ann Otol Rhinol Laryngol 1964;73:599-617.
9. Sprague JM. The innervation of the pharynx in the rhesus monkey and the formation of the pharyngeal plexus in primates. Anat Rec 1944;90:197-205.
10. Mu L, Sanders I. The innervation of the human upper esophageal sphincter. Dysphagia 1996;11:234-8.
11. Hollinshead WH. The head and neck. New York, NY: Hoeber-Harper, 1954:421-3. (Anatomy for surgeons; vol 1.)
12. Hwang K, Grossman MI, Ivy AC. Nervous control of the cervical portion of the esophagus. Am J Physiol 1948;154:343-57.
13. Rustard WH, Morrison LE. Revised anatomy of the recurrent laryngeal nerves: surgical importance based on the dissection of 100 cadavers. Laryngoscope 1952;62:237-49.
14. Steinberg JK, Khane GJ, Fernandes CMC, Nel JF. Anatomy of the recurrent laryngeal nerve: a redescription. J Laryngol Otol 1986;100:919-27.
15. Sjoberg AA. Experimental and clinical studies of paresis of the pharynx, larynx and oesophagus. Acta Otolaryngol [Suppl] (Stockh) 1939(suppl 38).
16. Rogers L. The treatment of spasmodic dysphagia by sympathetic denervation. Br J Surg 1935;22:829.
17. Kirchner JA. The motor activity of the cricopharyngeus muscle. Laryngoscope 1958;68:1119-59.
18. Murakami Y, Fukuda H, Kirchner JA. The cricopharyngeus muscle, an electrophysiological and neuropharmacological study. Acta Otolaryngol [Suppl] (Stockh) 1972(suppl 311).
19. Medda BK, Lang IM, Dodds WJ, et al. Correlation of electrical and contractile activities of the cricopharyngeus muscle in cat. Am J Physiol 1997;273:G470-G479.
20. Sethi RK, Thompson LL. The electromyographer's handbook. Boston, Mass: Little, Brown, 1989.

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LARYNGEAL CHEMODENERVATION: EFFECTS OF INJECTION SITE, DOSE, AND VOLUME

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A canine model was used to measure changes in laryngeal adductory pressure (LAP) following injections of vecuronium bromide, a short-acting neuromuscular blocking agent. At a constant volume, LAP was inversely related to the dose (and concentration) of vecuronium injected. At a constant dose (0.05 mg), LAP did not vary significantly over a wide range of injection volumes, from 0.05 to 0.50 mL. At a constant dose and volume, the site of injection was varied among the anterior, middle, and posterior vocal fold, the interarytenoid region, and the anterior contralateral vocal fold. Reduction in LAP was greatest ($p < .05$) for the posterior vocal fold injection site (78% reduction); less reduction was seen for the middle (54%) and anterior (52%) vocal fold and interarytenoid (43%) injection sites. These results have implications for laryngeal botulinum toxin injections, which are discussed.

KEY WORDS — botulinum toxin, laryngeal adductory pressure, laryngeal chemodenervation, spasmodic dysphonia, vecuronium bromide.

INTRODUCTION

Laryngeal chemodenervation with injections of botulinum toxin type A into the thyroarytenoid (TA) muscle is currently the treatment of choice for adductor spasmodic dysphonia.[1,2] Experience with these injections has led some clinicians to question whether the injections should be made into other laryngeal adductor muscles, such as the lateral cricoarytenoid (LCA). Others have suggested that the volume of injectate should be kept within a small range in order to optimize the diffusion of the toxin. To date, these questions have not been answered.

There is evidence that intramuscular botulinum toxin injections diffuse from the original site into nearby muscles. Such diffusion has been observed clinically in patients undergoing botulinum injections into facial muscles,[3] forearm muscles,[4] and cervical muscles.[5,6] The cervical injections for torticollis often affect swallowing; the eyelid injections for blepharospasm may cause temporary weakness of facial muscles resulting in an asymmetric smile. The effects of the toxin on these nearby muscles are shorter-lasting than the effects on the primary muscle injected, however.

A diffusion effect has also been advanced for botulinum toxin injected into the larynx.[7] George et al[8] demonstrated spread to the LCA muscle after injecting as little as 1.0 unit into the canine TA muscle. Shaari et al[9] found that the toxin easily spread through fascia planes, using a rat model. Borodic et al[10] found a diffusion gradient of denervation in a rabbit model; the magnitude and extent of this gradient were dose-dependent. The diffusion gradient concept would explain the shorter duration of action of the toxin on the noninjected nearby muscles. The physiological effects of TA injection diffusion to other laryngeal muscles have not been previously reported.

Vecuronium bromide is a water-soluble, short-acting (25 to 40 minutes), nondepolarizing (curariform) neuromuscular blocking agent. We have previously observed that intramuscular vecuronium injections can be used as a predictor of botulinum toxin effects. Injections into the TA muscle result in 15 to 30 minutes of relief of spasms in most patients with adductor spasmodic dysphonia. We have used these injections to assist with difficult diagnoses (eg, patients with atypical spasmodic dysphonia), to convince patients of the benefits of botulinum injections, to reassess patients who have failed more than 1 botulinum injection, and to determine which muscles to inject with botulinum (eg, in patients with mixed dystonias).

For the purposes of this study, the short-acting nature of vecuronium allows for complete recovery within a few hours, with no cumulative effect. Thus,

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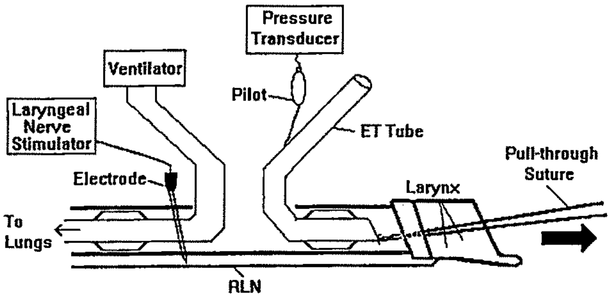

Fig 1. Schematic of experimental setup for laryngeal adductory pressure (LAP) measurements. Caudal endotracheal (ET) tube through tracheostomy connects lungs to ventilator. Rostral ET tube balloon is passed through vocal folds by pulling suture through mouth while stimulating recurrent laryngeal nerve (RLN). Pressure from vocal folds squeezing on balloon is measured on pressure transducer.

several experimental trials can be performed on the same animal within a reasonable period of time. Because of its similarity in clinical activity, the findings in this study with vecuronium should be analogous to those obtained with botulinum injections. To perform these experiments with botulinum toxin injections instead of vecuronium would be cost-prohibitive.

The canine larynx is very similar to the human larynx, with many analogous neuromuscular structures,[11] and makes an excellent model for this study.[12] In particular, the vocal folds are similar in size and shape, although the lamina propria is less well developed. The TA and LCA muscles have a similar relationship with one another, and the fascial planes around them are also analogous. This model makes it possible to study the effects of injection site, dose, and volume on the larynx without the need for human subjects, while still allowing reasonable conclusions to be drawn.

METHODS AND MATERIALS

Four purpose-bred, conditioned, female hound-type mongrel dogs weighing 20 kg were used for these experiments. They were housed in a facility approved by the American Association for Accreditation of Laboratory Animal Care, and National Institutes of Health guidelines for animal care were followed strictly. The experimental protocol was approved by the animal care committee of the St Louis Veterans Affairs Medical Center.

A permanent tracheostomy was performed according to our previously described method.[13] Briefly, general anesthesia was induced with intravenous thiopental sodium, and maintained with 1% to 2% halothane inhalant. An incision was made between tracheal rings 10 and 13. The anterior half of the tracheal ring cartilage was removed, leaving laterally based mucosal flaps. An ellipse of skin was excised to reduce skin redundancy, and the stoma was matured with deep stay sutures between the trachea and the dermis. The stoma was allowed to heal for 2 weeks.

Assessment of Vocal Fold Motion. Under general anesthesia, direct laryngoscopy was performed with Lewy arm suspension. A 4-mm telescope (Karl Storz) connected to a video camera (Panasonic KS152) was directed through the laryngoscope, and images were viewed on a monitor. Laryngeal activity was recorded on a videocassette recorder (Mitsubishi BV2000) for later analysis.

Recurrent laryngeal nerve (RLN) stimulation was performed by monopolar stimulation via either an implanted platinum electrode[12] (2 dogs) or a pertracheal Teflon-coated needle electrode[14] (2 dogs). The ground electrode was placed on the anterior chest wall. A custom constant-current laryngeal nerve stimulator (WR Medical Electronics) was set at 80 Hz. Vocal fold motion was observed on the monitor as the current was gradually increased. The stimulation currents at maximum and minimum levels of visible adductory activity were recorded. After successful placement of the pertracheal electrode, its position was maintained for the duration of the experiment. Care was taken to ensure that the monopolar current did not conduct to the opposite RLN; at the maximum stimulation level, no activity of the contralateral vocal fold was observed.

Laryngeal Adductory Pressure. The laryngeal adductory pressure (LAP) was measured according to the following method. Briefly, a 7.0-mm–inside diameter endotracheal (ET) tube was inserted through the tracheostomy and directed rostrally. The balloon was inflated with 10 mL of air, and the pilot line was connected to a pressure transducer (SpaceLab). A suture attached to the end of the ET tube and passed through the larynx and out the mouth assists in passing the balloon through the glottis. The gross LAP value was determined by passing the ET tube through the larynx while stimulating the RLN (Fig 1). The baseline level (measured with stimulator turned off) was subtracted to give the net LAP value. Measurements from 3 passes were averaged to give each data point. For each experiment, the LAP was measured at a series of stimulating currents, giving a sigmoid pressure response curve. The stimulating current level of the pressure plateau was usually slightly higher than the level of maximal observed activity.

Vocal Fold Injections. A 25-gauge needle on a tuberculin syringe was inserted percutaneously through the cricothyroid membrane while observing the monitor. The submucosal position of the needle could be determined by gentle motion of the syringe, and the

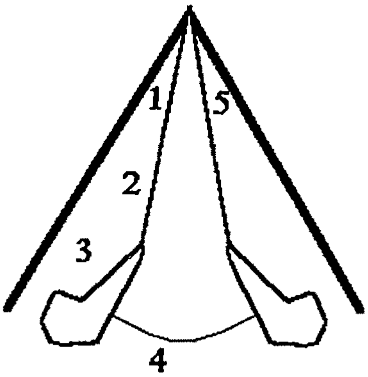

Fig 2. Five laryngeal injection sites: 1 — anterior vocal fold; 2 — mid–vocal fold; 3 — posterior vocal fold; 4 — interarytenoid; 5 — contralateral anterior vocal fold.

needle tip position ascertained. For these experiments, 5 injection sites were defined (Fig 2): 1) site 1, the anterior vocal fold, in the anterior third of the vocal fold; 2) site 2, the mid–vocal fold, halfway between the anterior commissure and the vocal process; 3) site 3, the posterior vocal fold, just posterolateral to the vocal process; 4) site 4, the interarytenoid site, in the posterior commissure at the midpoint between the corniculate cartilages; and 5) site 5, the contralateral vocal fold, anteriorly placed as in site 1.

Experimental Design. For each experiment, general anesthesia was induced as described above, and the laryngoscope and pertracheal electrodes were placed (or implanted electrodes accessed). The baseline LAP was measured and the optimal stimulation level was determined. A dose and volume of vecuronium bromide (Norcuron, Organon Inc) solution was prepared and injected according to one of the protocols below, and the time of injection was marked. The LAP was then measured serially at 5-minute intervals. Following injection of vecuronium, the LAP typically declined for several measurements, then slowly recovered to baseline over 1 to 2 hours. Measurements were continued until there was no further decline in LAP for 3 successive measurements, or longer. The animal was then disconnected from the apparatus and recovered from anesthesia. No more than 1 experiment was performed on any given day in a single animal, so that the injected vecuronium could be completely metabolized prior to the next experiment.

The following experiments were performed:

1. Constant volume, variable dose. The vecuronium bromide solution was varied so that an injection volume of 0.15 mL contained 0.025, 0.05, 0.10, 0.15, 0.20, or 0.30 mg. (The corresponding concentrations were 0.167, 0.333, 0.667, 1.0, 1.33, and 2.0 mg/mL, respectively.) Sterile water was used as the diluent. Control injections consisted of 0.15 mL of sterile water with no vecuronium. Injections were made into the middle of the vocal fold (site 2 above).

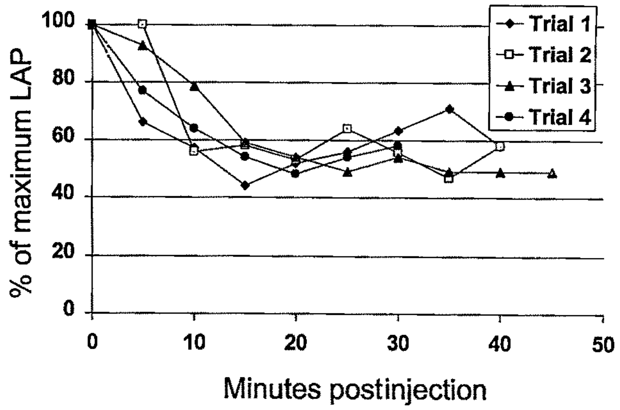

Fig 3. LAP versus time for 4 trials on different days of vecuronium 0.05 mg in 0.15-mL midcord injection. Good test-retest reliability is seen.

2. Constant dose, variable volume. We diluted 0.05 mg of vecuronium bromide with sterile water into total injection volumes of 0.05, 0.10, 0.15, 0.20, 0.30, and 0.50 mL (with concentrations of 1.0, 0.50, 0.333, 0.25, 0.167, and 0.10 mg/mL, respectively). Injections were made into the middle of the vocal fold.

3. Constant dose, constant volume, variable site. Injections of 0.15 mg of vecuronium in 0.15 mL of sterile water (1.0 mg/mL) were made at each of the 5 sites described above.

Each experiment described above was performed 2 to 5 times; the data from duplicate experiments were averaged. The LAP was expressed as a percentage of the baseline (maximum) value, and the series of LAP measurements was plotted against time postinjection. Thus, lower LAP values (as a percentage of maximum) indicate a greater effect of the vecuronium. The curves were compared with the paired *t*-test, and differences were considered significant if $p < .05$.

RESULTS

A total of 37 vecuronium injections were performed. There were no complications from the multiple anesthetics or from electrode implantation; however, electrode failure led to subsequent use of the pertracheal method in 2 dogs.

The vecuronium injections caused a characteristic reduction in LAP in all cases; no change in LAP was seen in the sterile water injection controls. The test-retest reliability was high, as seen in Fig 3. Similar results were obtained on different dates in the same dog, and among different dogs ($p < .05$).

In the 0.15-mL constant-volume series, increasing the dose of vecuronium resulted in a greater de-

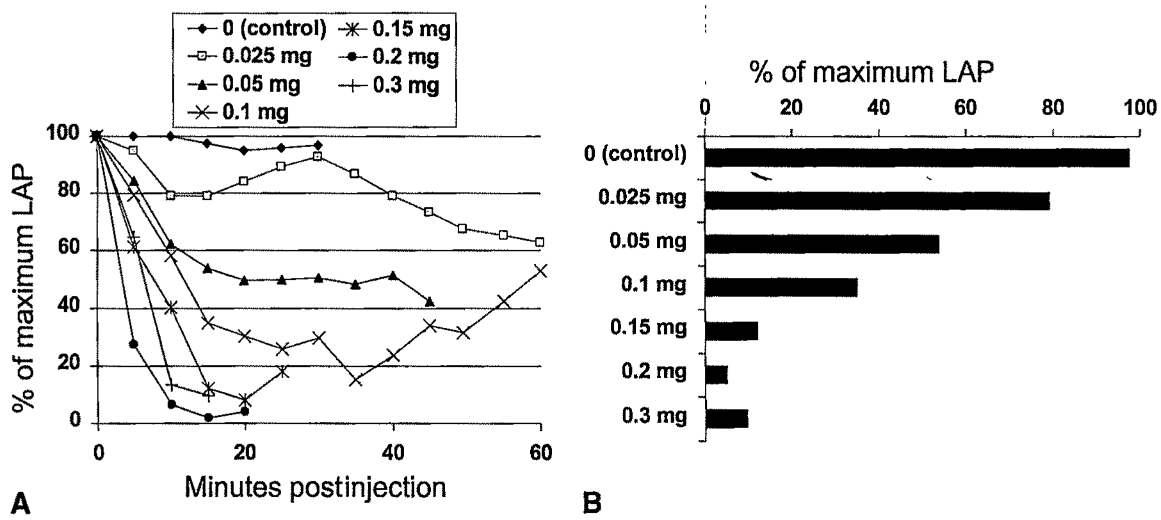

Fig 4. Results of constant-volume, variable-dose series. A) Reduction in LAP is directly related to quantity of vecuronium injected. B) This can be seen clearly by comparing LAP levels at single time point (15 minutes).

crease in LAP up to a dose of 0.15 mg; doses of 0.2 mg and 0.3 mg did not result in significant additional adductory weakness (Fig 4A). The initial decrease in LAP was faster (the slope was steeper) with higher doses than with lower doses, as seen in the degree of LAP reduction at 15 minutes (Fig 4B). Recovery to baseline activity was also slower with higher injection doses.

In the 0.05-mg constant-dose series, the LAP did not vary significantly with injection volume (Fig 5). There was a trend toward greater LAP reduction with higher volume, but it was not statistically significant. The initial slopes were nearly identical for all injection volumes tested.

In the constant-dose, constant-volume, variable-site-of-injection series, the greatest reduction in LAP was seen with the posterior injections ($p < .05$; Fig 6). Reductions in LAP for the middle and anterior vocal fold sites were similar to each other and were less than that for the posterior site. The interarytenoid site also showed LAP reduction, but the magnitude was smaller and the onset slower than for the vocal fold sites ($p < .05$). If the maximum decrease in LAP is considered (regardless of when it occurred, allowing an adjustment for different diffusion rates and distances), these data remain significant ($p < .05$; Fig 6B).

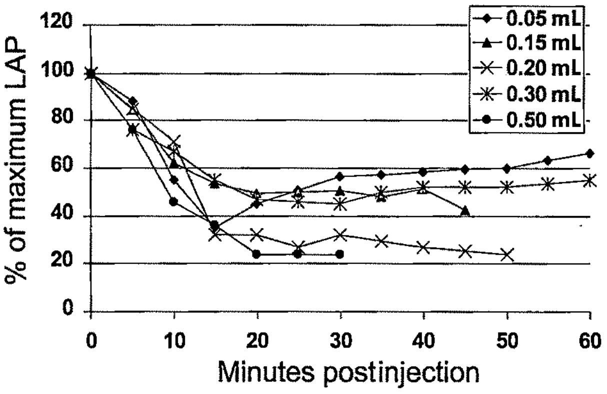

Fig 5. Results of constant-dose (0.05 mg), variable-volume series. LAP response is similar for all total injection volumes tested.

The contralateral anterior vocal fold injections gave inconsistent results. In 4 experiments, there was little or no effect in 2 trials, and reduction similar to that in the interarytenoid injection in the other 2, suggestive of slow diffusion of the vecuronium across the anterior commissure. Because of this inconsistency, the data were omitted from Fig 6.

DISCUSSION

The vecuronium gave a graded response that was dose-dependent, up to an apparent saturation level at about 0.15 mg. To the extent that vecuronium can be considered a model for botulinum toxin, this result would be consistent with the notion that either drug causes neuromuscular blockade by binding with cholinergic receptors, with the blockade increasing until some saturation point is reached. The duration of action of both drugs is a function of the tightness of this receptor binding, and of the degradation rate of the bound complexes. Botulinum toxin type A is a much larger molecule than vecuronium, and its tissue diffusion properties may differ; but our prior observations of similar activity in spasmodic dysphonia patients suggest that vecuronium is an adequate model for the purposes of this study.

The similar results with a variety of injection volumes indicate that the drug stays in the tissue and binds to the receptor sites quickly, before the remaining diluent (sterile water) can carry it away. This find-

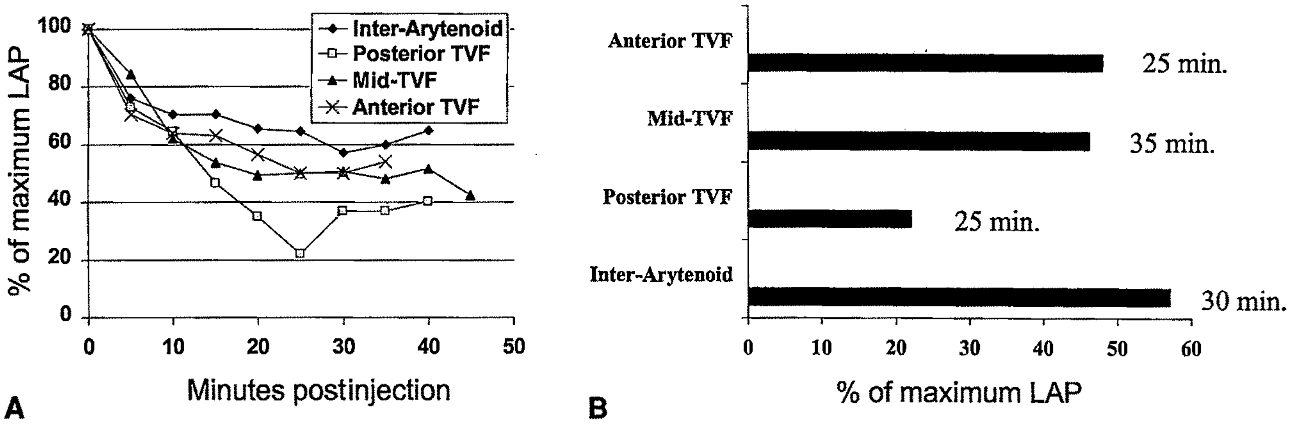

Fig 6. Results of constant-volume, constant-dose, variable-site-of-injection series. TVF — true vocal fold. A) LAP response versus time for 4 sites. B) Posterior vocal fold site gave greatest reduction. Time to peak activity varies, possibly due to different diffusion rates at each site.

ing has practical value in the administration of small doses of botulinum toxin to spasmodic dysphonia patients. It appears it is relatively safe to dilute the toxin to any suitable concentration to deliver the desired number of units (keeping the total injection volume within reason). Higher dilutions and larger injection volumes offer the advantage of more accuracy in administering the desired dose; a 0.01-mL error in injection volume is a greater error if the total volume is 0.05 mL than it is if the volume is 0.25 mL.

The site-of-injection series shows there is clearly a functional effect of diffusion of the injected vecuronium into nearby muscles. The posterior TA injection site, with the greatest reduction in LAP, probably included a significant diffusion into the nearby LCA muscle. The interarytenoid injection also probably diffused into the LCA muscle as well as the TA muscle; the reduction in LAP was more than could be explained by blocking the interarytenoid muscle alone. The variable effects found by moving the injection site along the long axis of the vocal fold suggest that small variations in site of injection might result in significant clinical variability in response to the botulinum; and this is clearly what we have observed in spasmodic dysphonia patients with the percutaneous injection technique. Although we confirm needle placement into the TA muscle using electromyography, an appropriate electromyographic signal might be obtained anywhere along this axis. Although these data clearly confirm diffusion of the injectate (vecuronium), they also support the findings of Borodic et al[10] that there is a diffusion gradient, with the majority of the drug's activity occurring at the site of deposition. Thus, it appears that the clinical response to botulinum injections might be more consistent if the injection needle can be reliably placed in the same vocal fold position every time.

The data from this study might also explain why some clinicians use consistently higher or lower doses of botulinum than others; it may be a function of where along the TA axis the drug is deposited. A more posterior placement is more likely to diffuse into the LCA muscle and to give a greater reduction in LAP. Would it be better to target the LCA muscle directly? Diffusion to the TA muscle would probably occur. One advantage of LCA injections might be to enforce consistency in the placement of the needle with each injection. Carefully controlled clinical studies are needed to ascertain this.

CONCLUSION

Intramuscular injections of vecuronium bromide cause a reduction in LAP that is dose-related. The volume of injection is not critical, within the tested range. The effect is greatest with a posterior vocal fold placement, probably from a higher degree of diffusion into the LCA muscle. Botulinum toxin probably has diffusion properties similar to those of vecuronium. If so, the data from this study can be used in choosing the site, dose, and volume of injection for patients undergoing laryngeal botulinum toxin injections.

REFERENCES

1. Blitzer A, Brin MF, Fahn S, Lovelace RE. Localized injections of botulinum toxin for the treatment of focal laryngeal dystonia. Laryngoscope 1988;98:193-7.

2. Blitzer A, Brin MF. Laryngeal dystonia: a series with botulinum toxin therapy. Ann Otol Rhinol Laryngol 1991;100: 85-9.

3. Eleopra R, Tugnoli V, Caniatti L, DeGrandis D. Botulinum toxin treatment in the facial muscles of humans: evidence of an action in untreated near muscles by peripheral local diffusion. Neurology 1996;46:1158-60.

4. Ross MH, Charness ME, Sudarsky L, Logigian EL. Treatment of occupational cramp with botulinum toxin: diffusion of

toxin to adjacent noninjected muscles. Muscle Nerve 1997;20: 593-8.

5. Buchman AS, Comella CL, Stebbins GT, Tanner CM, Goetz CG. Quantitative electromyographic analysis of changes in muscle activity following botulinum toxin therapy for cervical dystonia. Clin Neuropharmacol 1993;16:205-10.

6. Borodic GE, Joseph M, Fay L, Cozzolino D, Ferrante RJ. Botulinum A toxin for the treatment of spasmodic torticollis: dysphagia and regional toxin spread. Head Neck 1990;12: 392-9.

7. Kobayashi T, Niimi S, Kumada M, Kosaki H, Hirose H. Botulinum toxin treatment for spasmodic dysphonia. Acta Otolaryngol Suppl (Stockh) 1993(suppl 504):155-7.

8. George EF, Zimbler M, Wu B-L, Biller HF, Sanders I. Quantitative mapping of the effect of botulinum toxin injections in the thyroarytenoid muscle. Ann Otol Rhinol Laryngol 1992;101:888-92.

9. Shaari CM, George E, Wu BL, Biller HF, Sanders I. Quantifying the spread of botulinum toxin through muscle fascia. Laryngoscope 1991;101:960-4.

10. Borodic GE, Ferrante R, Pearce LB, Smith K. Histologic assessment of dose-related diffusion and muscle fiber response after therapeutic botulinum A toxin injections. Mov Disord 1994;9:31-9.

11. Berke GS, Moore DM, Hantke DR, Hanson DG, Gerratt BR, Burstein F. Laryngeal modeling: theoretical, in vitro, in vivo. Laryngoscope 1987;97:871-81.

12. Paniello RC, Dahm JD. Long-term model of induced canine phonation. Otolaryngol Head Neck Surg 1998;118:512-22.

13. Dahm JD, Paniello RC. Tracheostomy for long-term laryngeal experimentation. Otolaryngol Head Neck Surg 1998; 118:376-80.

14. Paniello RC, Lee P. Pertracheal stimulation of the recurrent laryngeal nerve. Arch Otolaryngol Head Neck Surg (in press).

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T CELLS INFILTRATING FROM THE SYSTEMIC CIRCULATION PROLIFERATE IN THE ENDOLYMPHATIC SAC

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It has been reported that autoimmune mechanisms are involved in the development of inner ear disorders such as Meniere's disease and steroid-responsive sensorineural hearing loss. In the present study, using an animal model for graft-versus-host disease, we investigated the immune regulatory mechanism in the endolymphatic sac and demonstrated that donor T cells injected into the systemic circulation of recipients infiltrate and proliferate in the perisaccular region. These findings suggest that immunocompetent cells are supplied from the systemic circulation through blood-labyrinth and blood-endolymph barriers into the endolymphatic sac, and that the endolymphatic sac allows these cells to proliferate locally as a local immune defense. It therefore seems likely that the endolymphatic sac plays a crucial role in not only graft-versus-host disease but also autoimmune inner ear disorders.

KEY WORDS — autoimmune disease, 5-bromo-2'-deoxyuridine, endolymphatic sac, graft-versus-host disease, immunocompetent cell.

INTRODUCTION

It has been reported that the endolymphatic sac is a major tissue in the inner ear, in which immunocompetent cells appear and show an immune reaction.[1,2] Using an animal model for acute graft-versus-host (GVH) disease, we have previously shown that immunocompetent cells are supplied from the systemic circulation into the endolymphatic sac.[3] McCabe[4] demonstrated the relationship between inner ear disease and immunologic disorders. Systemic autoimmune diseases such as rheumatoid arthritis,[5] relapsing polychondritis,[6] Behçet disease,[7] and systemic lupus erythematosus[8,9] have also been reported to be associated with inner ear disorders. Therefore, it is suggested that systemic autoimmune diseases cause dysfunction in the immune system of the inner ear.

Takahashi and Tomiyama[10] have demonstrated the proliferation of immunocompetent cells in the endolymphatic sac after immunostimulation using keyhole limpet hemocyanin. However, they have not demonstrated whether these cells are derived from the systemic circulation.

In the present study, we investigate the proliferative activity of the immunocompetent cells infiltrating the endolymphatic sac from the systemic circulation using 5-bromo-2'-deoxyuridine (BrdU), which is a thymidine analog and specifically incorporated into the nuclei of the cells in the S phase of the cell cycle.[11,12]

MATERIALS AND METHODS

Female BALB/c and C57BL/6 mice were purchased from CLEA Japan Inc (Osaka, Japan) and maintained under specific pathogen–free conditions in our animal facilities until use.

BALB/c mice with the H-2^d type of major histocompatibility complex (MHC) antigen were divided into 3 groups. The first group of mice (10 BALB/c) were irradiated with 8 Gy from a cesium 137 source (Gammacell 40 Exactor, Nordion International Inc, Kanata, Canada). One day later, these mice were injected intravenously with 7×10^7 splenic cells from C57BL/6 mice (H-2^b) after treatment with anti-B cell antibody (J11d2, ATCC, Rockville, Md) plus rabbit complement to delete B cells and to enrich T cells, resulting in induction of acute GVH disease. The second group of mice (10 BALB/c) were injected with the same number of lymphocytes from syngeneic BALB/c mice after irradiation. The third group of mice (10 BALB/c) were neither irradiated nor injected. The BALB/c mice in these 3 groups were then injected intraperitoneally with 10 µL BrdU (Amersham International, Little Chalfont, England) per gram of body weight on day 6. They were anesthe-

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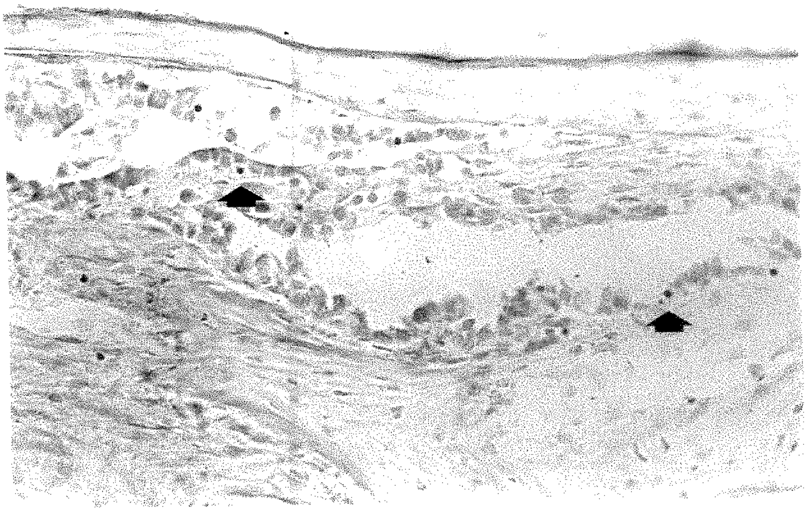

Fig 1. Mononuclear cells bearing H-2^b (donor) antigen in perisaccular region of rugose portion in [C57BL/6→BALB/c] mice (arrows; original ×400).

tized intraperitoneally with sodium pentobarbital (50 mg/kg) and subjected to intracardiac perfusion with saline 2 hours after BrdU inoculation, followed by periodate-lysine-paraformaldehyde (PLP) fixation at 4°C and decalcification at 4°C in 5% buffered ethylenediaminetetraacetic acid (EDTA) for 7 days. The temporal bones were frozen in OCT compound (Tissue-Tek II, Lab-Tek Products, Naperville, Ill), sectioned into 9-μm sections horizontally to the long axis of the cochlea, and then air-dried at room temperature.

The sections were applied immunohistochemically by a modified indirect immunoperoxidase technique[13] and were stained with hematoxylin and eosin. Several antibodies were employed: biotinylated anti-H-2^b antibody (Meiji, Tokyo, Japan) to stain the donor (C57BL/6) cells, biotinylated anti-H-2^d antibody (Meiji) to stain the host (BALB/c) cells, biotinylated anti-Thy1.2 antibody (Becton Dickinson, Mountain View, Calif) to stain T cells, and anti-IgG antibody (Kappel, Organon Teknika Corporation, North Carolina) to detect IgG-bearing cells. The avidin-biotin complex immunostaining method was applied with ABC kits (Vectastain, Burlingame, Calif). The sections were counterstained with hematoxylin.

To detect BrdU-positive cells, we stained the sections using the Cell Proliferation Kit (Amersham International) including murine anti-BrdU mononuclear antibody (IgG2a) and caprine peroxidase anti-mouse IgG2a antibody, followed by counterstaining with hematoxylin. The ovaries and brains from nontreated BALB/c mice were used as positive and negative controls, respectively.

RESULTS

Mononuclear cells that had infiltrated the perisaccular region of the rugose portion of the endolymphatic sac were positively stained with anti-H-2^b antibody (Fig 1) and with anti-Thy1.2 antibody (Fig 2) in all 10 of the BALB/c mice that received spleen cells from allogeneic C57BL/6 mice [C57BL/6→BALB/c]. However, these cells were not stained with

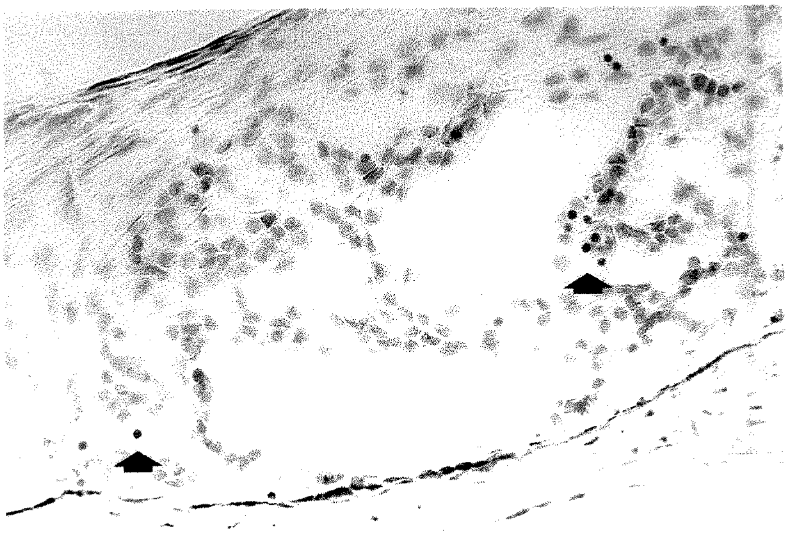

Fig 2. Mononuclear cells bearing Thy1.2 (T cell) antigen in perisaccular region of rugose portion in [C57BL/6→BALB/c] mice (arrows; original ×400).

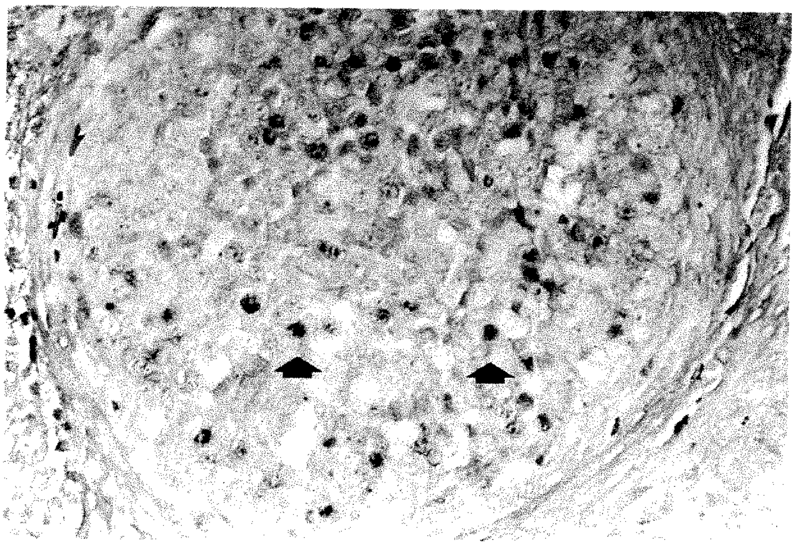

Fig 3. Cells with BrdU-positive nuclei in ovary tissue of BALB/c mice (arrows; original ×300).

anti-H-2^d antibody on the cell surface (data not shown). The BALB/c mice, which had received the spleen cells from syngeneic BALB/c mice [BALB/c→BALB/c], showed no cell infiltration in the perisaccular region (data not shown). No cell infiltration was seen in other areas, including the modiolar vein in the inner ear of all groups of mice (data not shown).

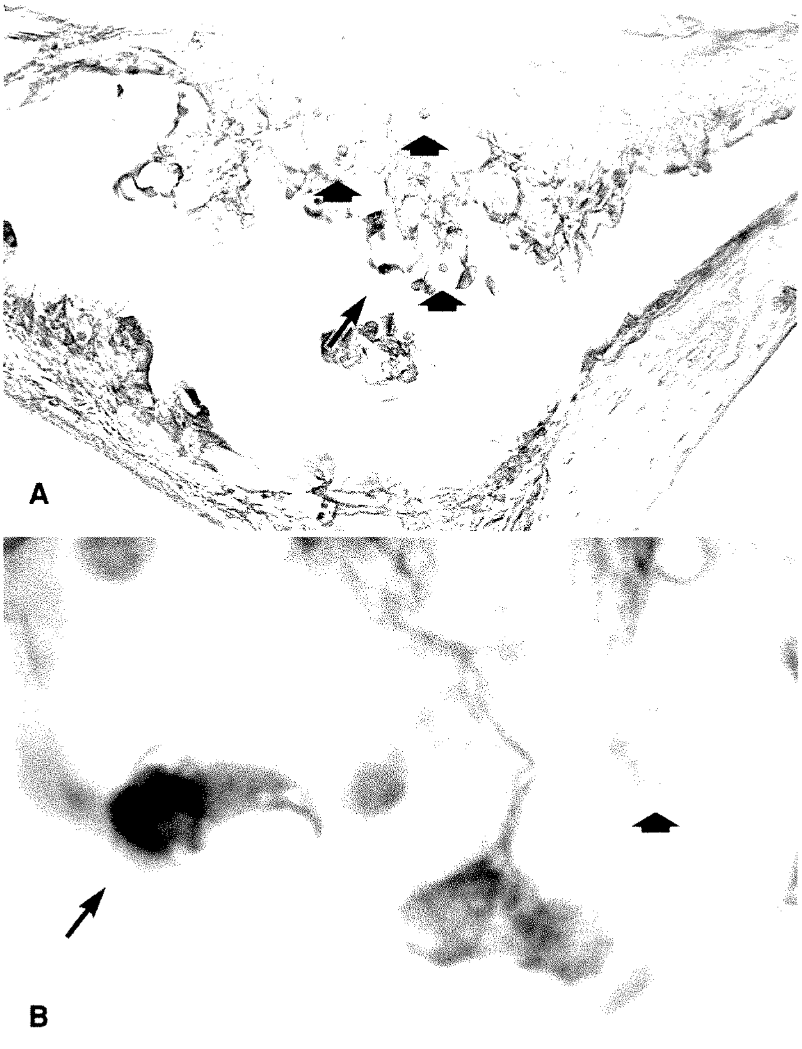

Fig 4. A) BrdU-positive nucleus (long arrow) and BrdU-negative infiltrated cells (short arrows) in perisaccular region of rugose portion in [C57BL/6→BALB/c] mice (original ×400). B) High magnification of A showing enlargement and irregularity of BrdU-positive nucleus corresponding to S phase (original ×2,000).

The next step was to examine cell proliferation by means of BrdU. In the BALB/c mice (without irradiation), the ovary tissue showed BrdU-incorporated nuclei (Fig 3), while the brain tissue contained no BrdU-positive nuclei (data not shown). The cells that had infiltrated the perisaccular region in 8 of the 10 [C57BL/6→BALB/c] mice also showed positive staining with anti-BrdU antibody (Fig 4A). The nuclei showed enlargement and irregularity corresponding to the S phase of the cell cycle (Fig 4B). From 1 to 3 of the cells infiltrating each endolymphatic sac were positively stained with anti-BrdU antibody.

The IgG-positive cells were also examined. However, no IgG-bearing cells (including IgG2a-bearing cells) were observed in the perisaccular region of the [C57BL/6→BALB/c] mice or in the ovaries of the BALB/c mice (data not shown).

DISCUSSION

Regarding the possibility of cell infiltration around the modiolar vein and other areas of the inner ear, we could find cell infiltration only in the perisaccular region. It is well known that allogeneic T cells cause acute GVH disease in immunologically active sites,[14] and that the endolymphatic sac is a major immune tissue and an immunologically active and antigenic site in the inner ear.[1,2,15] This is, therefore, the reason why acute GVH disease occurs only in the perisaccular region of the inner ear. We have reported that acute GVH disease occurs in the endolymphatic sac.[3] Various investigators have demonstrated that immunocompetent cells reach the endolymphatic sac from the systemic circulation.[3,16,17] Takahashi and Tomiyama[10] reported that immunocompetent cells in the endolymphatic sac showed proliferative activity upon antigen exposure. However, it remained uncertain whether immunocompetent cells in the systemic circulation could infiltrate and proliferate sequentially in the endolymphatic sac. In the present study, we have demonstrated, using an animal model for GVH disease, the existence of BrdU-carrying cells in the donor T cells infiltrating the perisaccular region. BrdU is a thymidine analog specifically incorporated into the nuclei of the cells in the S phase — the period of DNA synthesis — of the cell cycle.[11,12] These findings suggest that immunocompetent cells in the systemic circulation infiltrate the endolymphatic sac through the blood-labyrinth and blood-endolymph barriers and proliferate locally as a consequence of the immune response.

We used the Cell Proliferating Kit (Amersham), which includes caprine peroxidase anti-mouse IgG2a antibody as a second antibody, to detect BrdU-positive cells. This antibody directly detects mouse IgG2a on the murine sections. However, no IgG-bearing cells, including IgG2a-positive cells, were observed in the perisaccular region.

A previous study showed that some epithelial cells and fibroblast cells proliferated in the perisaccular region of the guinea pig.[18] In the present study, the [BALB/c→BALB/c] mice did not show any proliferating cells in the perisaccular region, whereas the [C57BL/6→BALB/c] mice did. Since host BALB/c mice were irradiated with 8 Gy, this treatment appears to inhibit the proliferation of these cells in the host perisaccular region. Therefore, it is likely that BrdU-positive cells are donor cells that have infiltrated this region, rather than host-derived cells, including epithelial cells or fibroblast cells, in the perisaccular region of the [C57BL/6→BALB/c] mice. In the present study, we have shown that there is an immunologic communication between the systemic circulation and endolymphatic sac, although the endolymphatic sac is surrounded by blood-labyrinth and blood-endolymph barriers. Autoimmune diseases have been reported to be associated with inner ear disorders.[5-9]

It has also been reported that there is a close correlation between autoimmune disease and GVH disease; Gleichmann et al[19] have demonstrated that autoimmune disease can be induced by GVH disease elicited by injection of parental lymphocytes into F1 mice. It therefore seems likely that systemic autoimmune diseases like GVH disease affect the inner ear immune function and give rise to inner ear disorders. Indeed, some forms of Meniere's disease[4,20] and steroid-responsive sensorineural hearing loss[21,22] have been suggested to be autoimmune diseases. It has been demonstrated that there was infiltration of T cells in the perisaccular region of a patient with Meniere's disease,[23] as is seen in patients with Hashimoto's thyroiditis or insulin-dependent diabetes mellitus. From our present data showing that immunocompetent cells in the systemic circulation infiltrate and proliferate in the endolymphatic sac, it appears likely that the endolymphatic sac is also a target site of autoimmune disease.

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REFERENCES

1. Lim D, Silver P. The endolymphatic duct system. A light and electron microscopic investigation [Abstract]. In: Pulec J, ed. Proceedings of the Barany Society Meeting. Los Angeles, Calif: 1974:390.

2. Rask-Andersen H, Stahle J. Immunodefense of the inner ear? Lymphocyte-macrophage interaction in the endolymphatic sac. Acta Otolaryngol (Stockh) 1980;89:283-94.

3. Iwai H, Tomoda K, Inaba M, et al. Evidence of cellular supplies to the endolymphatic sac from the systemic circulation. Acta Otolaryngol (Stockh) 1995;115:509-11.

4. McCabe BF. Autoimmune sensorineural hearing loss. Ann Otol Rhinol Laryngol 1979;88:585-9.

5. Goodwill CJ, Lord IJ, Jones RPK. Hearing in rheumatoid arthritis. Ann Rheum Dis 1972;31:170-3.

6. Cody D, Tones DA. Relapsing polychondritis; audiovestibular manifestations. Laryngoscope 1971;81:1208-22.

7. Brama I, Fainaru M. Inner ear involvement in Behçet's disease. Arch Otolaryngol 1980;106:215-7.

8. MacRae D, O'Reilly S. On some neuro-oto-ophthalmological manifestations of systemic lupus erythematosus and polyarteritis nodosa. Eye Ear Nose Throat Mon 1975;36:721-6.

9. Hamblin TJ, Mufti GJ, Bracewell A. Severe deafness in systemic lupus erythematosus: its immediate relief by plasma exchange. BMJ 1982;284:1374.

10. Takahashi M, Tomiyama S. Cell proliferation in the endolymphatic sac in situ after inner ear immunostimulation. Acta Otolaryngol (Stockh) 1995;115:396-9.

11. Gratzner HG. Monoclonal antibody to 5-bromo and 5-idodeoxyuridine: a new reagent for detection of DNA replication. Science 1982;218:474-5.

12. Harms G, van Goor H, Koudstaal J, de Lay L, Hardonk MJ. Immunohistochemical demonstration of DNA-incorporated 5-bromodeoxyuridine in frozen and plastic embedded sections. Histochemistry 1986;85:139-43.

13. Takahashi M, Harris JP. Anatomic distribution and localization of immunocompetent cells in normal mouse endolymphatic sac. Acta Otolaryngol (Stockh) 1988;106:409-16.

14. von Boehmer H, Sprent J. T-cell function in bone marrow chimeras: absence of host reactive T-cells and co-operation of helper T-cells across allogeneic barriers. Transplant Rev 1976;29:3-23.

15. Kawauchi H, Ichimiya I, Kaneda N, Mogi G. Distribution of immunocompetent cells in the endolymphatic sac. Ann Otol Rhinol Laryngol Suppl 1992;101(suppl 157):39-47.

16. Gloddek B, Ryan AF, Harris JP. Homing of lymphocytes to the inner ear. Acta Otolaryngol (Stockh) 1991;111:1051-9.

17. Rask-Andersen H. The vascular supply of the endolymphatic sac. Acta Otolaryngol (Stockh) 1979;88:315-27.

18. Takahashi M, Hokunan K, Unno T. S-phase cells in the guinea pig endolymphatic sac. Acta Otolaryngol (Stockh) 1994; 114:259-63.

19. Gleichmann E, Pals ST, Rolink AG, Radaszkiewicz T, Gleichmann H. Graft-versus-host reactions: clues to the etiopathology of a spectrum of immunological diseases. Immunol Today 1984;5:324-32.

20. Shea JJ. Autoimmune sensorineural hearing loss as an aggravation factor in Meniere's disease. Adv Otorhinolaryngol 1983;30:254-7.

21. Brookes GB, Newland AC. Plasma exchange in the treatment of immune complex-associated sensorineural deafness. J Laryngol Otol 1986;100:25-33.

22. Kanzaki J, O-uchi T. Circulating immune complex in steroid-responsive sensorineural hearing loss and the long-term observation. Acta Otolaryngol [Suppl] (Stockh) 1983(suppl 393):77-84.

23. Danckwardt-Lillieström N, Friberg U, Kinnefors A, Rask-Andersen H. "Endolymphatic sacitis" in a case of active Meniere's disease. An ultrastructural histopathologic investigation. Ann Otol Rhinol Laryngol 1997;106:190-8.

Ann Otol Rhinol Laryngol 108:1999

GEOMETRIC STRUCTURE OF THE HUMAN AND CANINE CRICOTHYROID AND THYROARYTENOID MUSCLES FOR BIOMECHANICAL APPLICATIONS

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The geometric structure of the cricothyroid (CT) muscle and thyroarytenoid (TA) muscle was quantified in 6 human and 3 canine larynges. Each muscle was divided into a series of fiber bundles. With a 3-dimensional micrometer probe, the coordinates of the origin and insertion of each bundle were measured before dissection. It was found that the mass of the CT muscle in the dog was 1.463 ± 0.280 g, which was significantly greater than the 0.9423 ± 0.123 g found in the human. This was a result of the cross-sectional area of the canine CT muscle being 105.3 ± 11.6 mm^2 instead of the 73.8 ± 7.4 mm^2 found for the human. However, the ratios of CT/TA mass and cross-sectional area between the two groups were not significantly different, suggesting that the two muscles grow proportionally.

KEY WORDS — canine larynx, cricothyroid muscle, human larynx, thyroarytenoid muscle.

INTRODUCTION

The intrinsic muscles of the larynx are essential in the control of loudness, pitch, and voice quality in speaking and singing. These muscles are part of a cooperative system used for the purpose of widening the glottis (abduction), closing the glottis (adduction), and lengthening the vocal folds. The mechanism of lengthening the vocal folds is of particular interest in this study; therefore, two intrinsic muscles, the cricothyroid (CT) and thyroarytenoid (TA) muscles, were chosen for a comparative analysis of fiber orientation and size. These two muscles are responsible for most mechanical actions within the larynx that affect pitch control.

Because the TA and CT muscles are in direct opposition to each other, they are used differentially as regulators of fundamental frequency (F0).[1] Many studies have clarified the function of the CT muscle in phonation by means of electromyography,[2-5] morphological techniques,[6] and experiments on dogs.[7-9] These studies indicate that increased CT activity raises F0, all else being constant. The CT muscle usually acts in a "non-isometric" mode and decreases its length during contraction. This decrease of length could reach up to 50% at high pitches.[10,11] Further, in the singer's chest voice, the activity of the CT muscle is increased with F0, and only in falsetto register does the activity reach its maximum.[3,10]

The function of the TA muscle, however, is less clear in F0 control. Increased activity of the CT muscle raises the F0, whereas increased activity in the TA muscle may either raise or lower the F0.[4,5,9,12] The tension of the vocal fold is controlled by the vocal fold length. Greater contraction of the muscle will lower the F0, because the active tension in the TA muscle is outweighed by the reduced tension in the cover due to the decrease in length.

The CT and TA muscles work together as agonist-antagonist pairs[10,13] as they cause rotation and gliding around the CT joint. In order to solve the dynamics of this rotation and gliding, it is necessary to know the net clockwise and counterclockwise torques that are produced by the contractions of both the CT and TA muscles. Their individual torque depends on the magnitude of force, the line of action, and the moment arm. If it is assumed that muscle bundles are activated equally, then the force can be proportional to the cross-sectional area of the muscle, and the effective torque can be calculated from the individual bundle's geometry (coordinates of origin, insertion, and cross-sectional area). Thus, the knowledge of muscular fiber length and orientation is an essential in any estimation of the torque and moment arm of CT rotation.

From the National Center for Voice and Speech and the Department of Speech Pathology and Audiology, The University of Iowa, Iowa City, Iowa. This work was supported by the National Institutes of Health/National Institute on Deafness and Other Communication Disorders (grant #P60 DC00976). This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of The University of Iowa (October 22, 1993).

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A comparative analysis between the anatomy of the human larynx and that of the canine larynx is important when studying these muscles, because much of the knowledge we gain about the vocal mechanism is obtained from animal experimentation. The canine larynx has been a popular substitute for the human larynx in a variety of studies because of its similar size and gross structure. It is critical, however, to identify differences that occur between the two species before application can be made to human function.

The purpose of this study was to examine the differences in orientation, length, mass, and cross-sectional area between the canine and human larynges and discuss their implications for pitch control. This will be done mainly in terms of ratios between CT and TA measures, which are most helpful for identifying differences in overall function.

METHOD

Human and Canine Tissue Collections. Male human larynges from autopsy were obtained from the Department of Pathology at the University of Iowa or from the University of Utah. The 6 larynges were collected and slowly frozen 5 to 19 hours after death and after the stages of rigor mortis in the neck region were completed. Their ages were 36, 65, 74, 78, 41, and 71 years. All larynges were obtained from individuals with no history of head or neck trauma and no laryngeal disease. The canine larynges were obtained from the University of Iowa Animal Care Unit after the dogs were painlessly sacrificed for experimentation in a cardiovascular research laboratory. The 3 specimens, from male dogs, were obtained immediately after death and submerged in 0.9% saline solution. The ages of the specimens were estimated by a University veterinarian on the basis of dental development and were found to be no more than 1 to 2 years. All specimens were from mixed Labrador and German shepherd breeds ranging in size from 25 to 26.1 kg. All larynges were obtained from dogs with no evidence of head or neck trauma or disease of the larynx.

Preparation of Tissue. The human larynges were frozen slowly following autopsy and stored for several days in a freezer (–4°C). They were also thawed slowly at room temperature before measurement. In order to minimize the effects of dehydration, the fluid balance of the dead tissue was maintained by keeping the larynx submerged in a 0.9% saline solution at room temperature before measurement. Throughout the dissection procedure and after each fiber was removed, the saline solution was applied to maintain hydration of each muscle and all dissected fibers. To prevent excessive hydration, the fiber was placed on absorbable paper for a brief moment to soak up surface saline before being weighed. The remaining tissue was submerged in 0.9% saline solution and stored in a refrigerator (+4°C) after each muscle was dissected.

The canine larynges were also submerged in 0.9% saline solution following removal and stored in a refrigerator (+4°C) for 20 to 48 hours before measurement. During dissection, the canine larynges were protected against dehydration via the same procedure used for the human larynges. Thus, the processes used immediately after harvesting were different for canine and human samples (freezing in human), but the means of storage during dissection were similar.

Fiber Dissection. Universal safety precautions were used for all dissection procedures. Microdissection scissors, lightweight sandpapers, scalpels, and tweezers were used to remove unwanted fat, fascia, and nerve tissue. The excised larynges were prepared such that only the major cartilages (thyroid, cricoid, arytenoid, and a piece of the trachea) and intrinsic muscles remained as the framework. Excess tissue was removed to expose the CT muscle, in particular, the pars recta (PR) and the pars obliqua (PO). Superiorly, all the tissue was removed to the level of the true vocal folds. The vocal fold mucosa and ligament were also removed so that all the TA muscle fibers were visible. To minimize tissue alterations, the same person performed all fiber dissections.

The second step in this procedure was to mount the larynx on a laboratory bench such that the cricoid cartilage was fixed. Rotation of the thyroid cartilage allowed the axis of rotation (through the CT joint) to be determined. A pin was inserted through both CT joints to establish the origin of the coordinate system and to keep the joints from rotating and slipping. The CT joint constituted one of the primary reference points. The midline of the lower border of the anterior arch of the cricoid cartilage constituted the second primary reference point, and the anterior commissure constituted the third primary reference point. A pin was also inserted through the cricoarytenoid joint to keep the arytenoid from sliding or rocking in any direction. The 3-dimensional geometry of the larynx was quantified with a 3-dimensional mechanical positioning system composed of 3 orthogonally mounted micrometers. Vernier markings allowed the position of each slide to be read to an accuracy of 0.1 mm. The range of measurement was determined so that the larynx could remain in a fixed position until all the data for that muscle were collected. Several additional reference points on the major cartilages (thyroid, cricoid, and arytenoid) were recorded with the device to indicate where the ori-

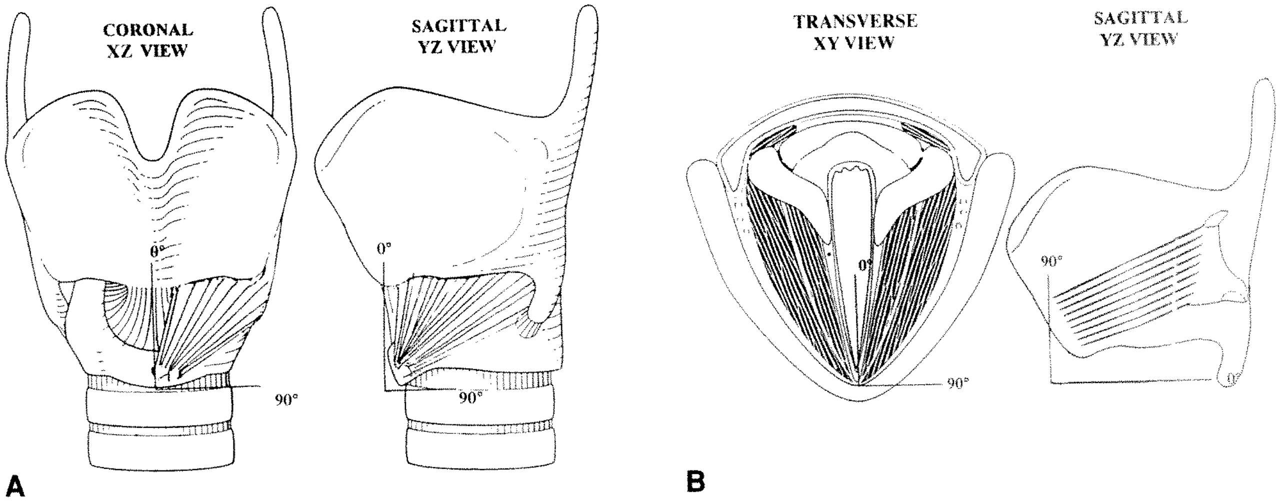

Fig 1. Views of A) thyroarytenoid (TA) muscle and B) cricothyroid (CT) muscle.

gins and insertions were in reference to the whole laryngeal framework. It should be noted that the left CT muscle and the right TA muscle were dissected for each specimen. Ease of dissection and apparatus limitations were the primary reasons that muscles of the same side were not used for each specimen. Symmetry was thus assumed in each case for analysis, but a test for symmetry in terms of bulk mass was performed (see below).

The third step in this procedure was to isolate fiber bundles on two separate portions of the CT muscle (PR and PO) and on the TA muscle (vocalis and muscularis). The vocalis and muscularis portions were not easily defined; therefore, there was no distinction made between the two portions in the TA. Muscle fiber bundles were defined by removing the thin layer of fascia and gently rubbing the muscle across the grain to separate the various fascicles. The fiber bundles were removed according to muscle definition and ease of separation. Superficial layers were separated and removed first to expose deeper layers of bundles. Three-dimensional coordinates of origin (X1, Y1, Z1) and insertion (X2, Y2, Z2) were recorded for each fiber bundle.

Mass Measurement. Each dissected muscle fiber bundle was weighed on an electronic balance (Mettler model AE 100, 0.1-mg accuracy) after removal from the muscle. The mass of each muscle fiber bundle was recorded and accumulated to represent the total muscle mass of the CT muscle, the PR and PO bellies of the CT muscle, and the TA muscle. The contralateral muscles were also weighed on the balance and recorded for 5 of the 6 human larynges and all 3 canine larynges.

Length and Angle Calculations. The length of each dissected muscle fiber bundle (L) was determined from the 3-dimensional coordinates of origin and insertion points as

$$L = \sqrt{(X2 - X1)^2 + (Y2 - Y1)^2 + (Z2 - Z1)^2}$$

After the lengths were recorded for each muscle fiber, the average length was determined for the complete muscle. The angle θ of each muscle bundle in the XY plane relative to the midsagittal plane was calculated from

$$\theta = \tan^{-1}\left(\frac{Y2 - Y1}{X2 - X1}\right)$$

In other planes, such as the YZ plane, it was calculated similarly (Fig 1). Then the range of angles for the complete muscle and its average were calculated for the CT muscle, TA muscle, PR, and PO.

Area Calculation. The cross-sectional area of each bundle, A, was calculated from

$$A = \frac{m}{\rho L}$$

where m is the bundle mass, ρ is the density of muscle tissue, and L is the bundle length. The areas of each muscle fiber bundle were recorded and added together to represent the total muscle area of the complete muscle.

Statistical Analysis. The CT muscle, PR, PO, and TA muscle were measured with respect to average length, total muscle mass, and total cross-sectional area of the muscle. To account for the discrepancies in the samples' geometric structure and make appropriate between-group comparisons, a nonparametric statistical test with the level of significance value of $p = .05$ was used (Mann-Whitney U test[14]). It compared these muscles in humans and dogs on these characteristics: 1) muscle mass, 2) CT/TA mass ratio, 3) muscle length, 4) muscle cross-sectional area, 5) CT/TA area ratio, and 6) orientation angle.

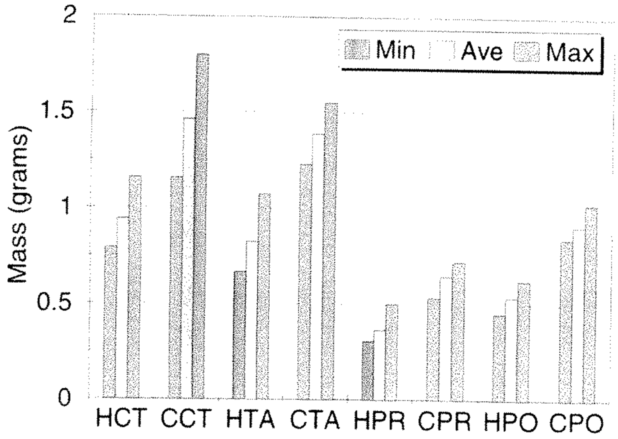

Fig 2. Comparative masses of CT and TA muscles. HCT — human cricothyroid muscle, CCT — canine cricothyroid muscle, HTA — human thyroarytenoid muscle, CTA — canine thyroarytenoid muscle, HPR — human pars recta, CPR — canine pars recta, HPO — human pars obliqua, CPO — canine pars oblique.

RESULTS

Comparative Mass Results. The total masses of the CT muscle, its PR and PO bellies, and the TA muscle were measured for the 6 human larynges and the 3 canine larynges. The total mass of the contralateral muscle was also measured for 5 of the human larynges and all 3 canine larynges. Figure 2 shows that the average total mass of the human CT muscles (denoted HCT on the bar graph) was 0.9423 g (SD = 0.1228 g). The average total mass of the canine CT muscles (denoted CCT) was 1.4632 g (SD = 0.2802 g). The results of the Mann-Whitney U test revealed significant between-group differences ($p = .0155$) in total mass for the CT muscles. Therefore, the canine CT muscles used in this study (large mongrel dogs) had about 0.5 g more mass than the human CT muscles. Similarly, the canine TA muscles had about 0.56 g more mass than the human TA muscles ($p = .001$).

Comparative Length Results. The average lengths of the CT muscle, its PR and PO bellies, and the TA muscle were calculated for the 6 human larynges and the 3 canine larynges. Figure 3 shows that the average length of the human CT muscles was 13.8 mm (SD = 1.0 mm). The average length of the canine CT muscles was 15.2 mm (SD = 0.4 mm). There is a significant between-group difference ($p = .0478$) in average length for the CT muscles; the canine CT muscles used in this study were longer than the human CT muscles by about 1 to 2 mm. Similarly, the canine TA muscles were about 3.6 mm longer than the human TA muscles ($p = .0476$).

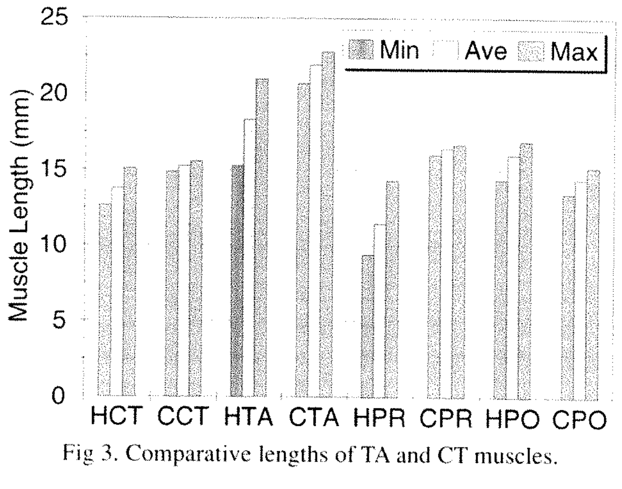

Fig 3. Comparative lengths of TA and CT muscles.

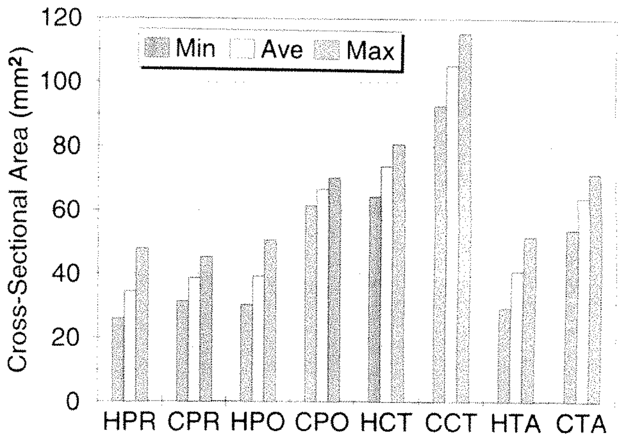

Fig 4. Comparative cross-sectional areas of TA and CT muscles.

Comparative Cross-sectional Area Results. Figure 4 shows that the average cross-sectional area of the human CT muscles was 73.8 mm^2 (SD = 7.4 mm^2) and the average cross-sectional area of the canine CT muscles was 105.3 mm^2 (SD = 11.6 mm^2). There was a significant between-group difference ($p = .0238$) in total muscle cross-sectional area for the CT muscle, with the canine CT cross-sectional area being about 30 mm^2 greater than the human. Similarly, the canine TA muscle, with an average cross-sectional area of 63.8 mm^2, was about 50% thicker than the human

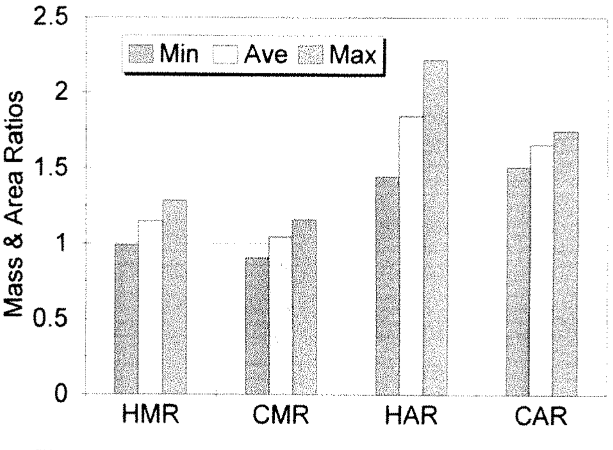

Fig 5. Comparative masses and area ratios of TA and CT muscles. HMR — human mass ratio, CMR — canine mass ratio, HAR — human area ratio, CAR — canine area ratio.

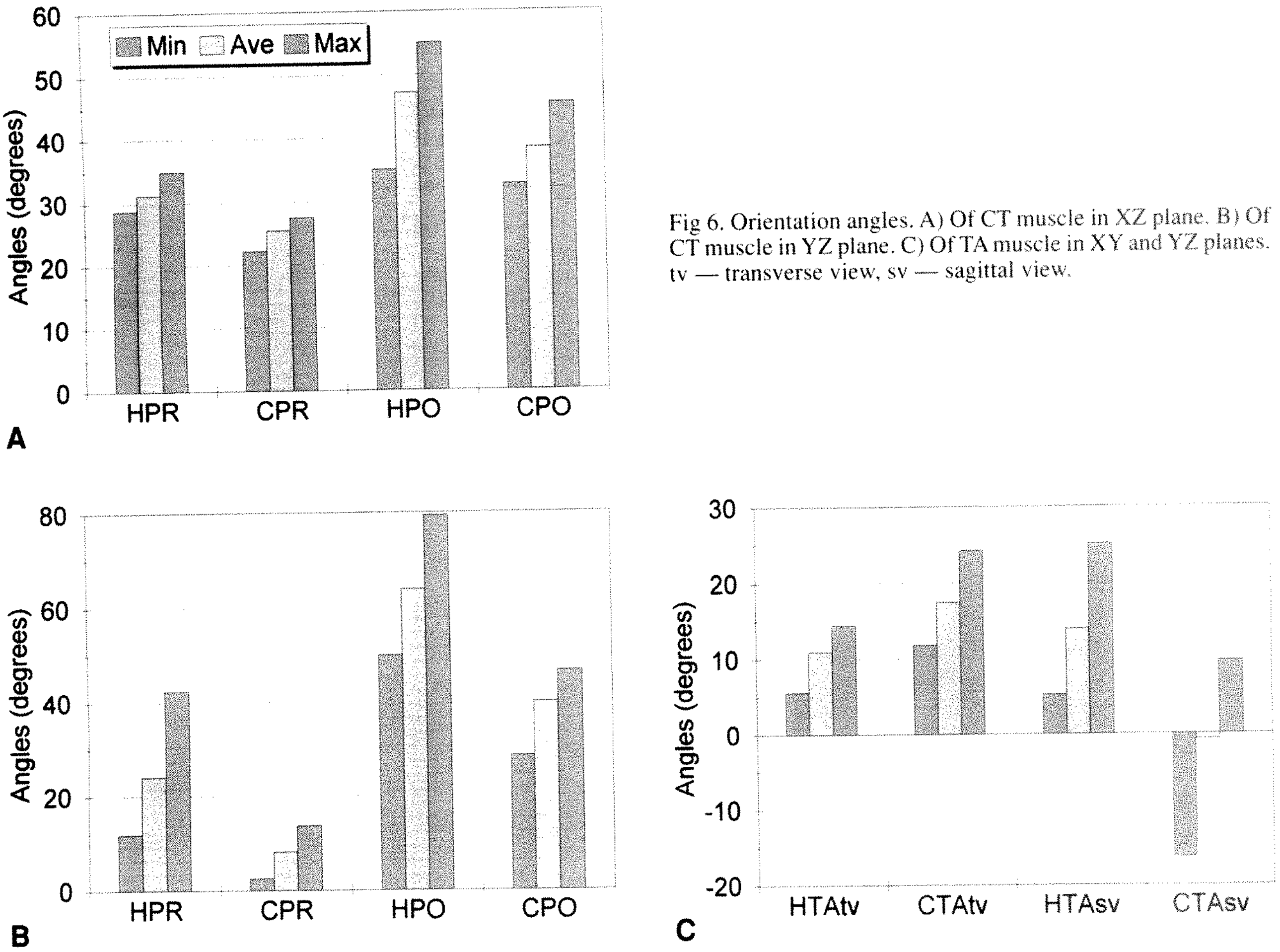

Fig 6. Orientation angles. A) Of CT muscle in XZ plane. B) Of CT muscle in YZ plane. C) Of TA muscle in XY and YZ planes. tv — transverse view, sv — sagittal view.

TA muscle, with a 40.9-mm^2 cross-sectional area (p = .0238).

Comparative Mass and Cross-sectional Area Ratios. The ratios of total CT muscle mass to total TA muscle mass were calculated to eliminate the effects of muscle bulk and atrophy due to the age of each specimen. Ratios were also useful in comparing the total masses of the two agonist-antagonist muscle pairs within each larynx. Mass ratios were calculated for the 6 human larynges and the 3 canine larynges. The ratios of the contralateral muscles were also calculated for 5 of the human larynges and the 3 canine larynges. Figure 5 shows that the average CT/TA mass ratio of the human muscles was 1.10506 (SD = 0.0999). The ratio for the dog was 1.0466 (SD = 0.1078); hence, there is no significant between-group difference (p = .119). Cross-sectional area ratios were also calculated for the 6 human larynges and the 3 canine larynges and are shown in the same Figure. The CT/TA cross-sectional area ratio of the human muscles was 1.8 (SD = 0.3). This ratio for the dog was 1.7 (SD = 0.1); there is no significant between-group difference (p = .381).

Comparative Angle Results. Figure 6A shows that the average orientation angle (from vertical position) of the PR belly of the human CT muscle was 31.2° (SD = 2.5°) in the XZ plane (coronal). The same angle for the dog was 25.5° (SD = 2.8°), with a significant between-group difference (p = .0238) of about 5° to 6°. This means that the canine CT muscle lies more vertical. The PO bellies of humans and dogs had average values of 47.2° (SD = 9.3°) and 38.3° (SD = 6.6°) in the same plane; ie, the human PO muscle is more horizontal than the canine.

These muscles have different angles in the YZ plane (midsagittal). Figure 6B shows that the PR belly had an average angle of 24.1° for human samples and 8.2° for canine samples. This is a significant difference (p = .0476) that indicates the canine PR muscle is more vertical in the midsagittal plane than the human PR muscle. These angles for the human and canine PO bellies were 63.9° and 40°; hence, the human PO is more horizontal in the YZ plane.

Figure 6C shows the average angles of the TA muscles of the human and canine samples. These angles are based on the measurements in the XY plane (transverse view) and the sagittal YZ plane as shown in Fig 1B. The average angle for the human TA muscle was 11.0° (SD = 3.3), and that for the canine TA muscle was 17.4° (SD = 6.3); thus, the canine vocal folds at equilibrium position are more abducted than human vocal folds. The average TA angles in the sagittal plane were 13.9° and −0.8°, so the canine TA muscle is almost horizontal, but the human TA muscle is tilted upward from anterior to posterior. However, these TA angle differences were not statistically significant.

DISCUSSION

Mass and cross-sectional area were significantly larger in our canine larynges than in our human larynges. However, the mass and cross-sectional area ratios show that the relationship between these two muscles is similar in the two species. If we assume that all the muscle bundles are equally activated, the force produced by each bundle is proportional to its cross-sectional area. Studying the mass and cross-sectional area ratios allows us to predict that the CT muscle is capable of producing greater forces in the elongation and rotation processes.[11] Cross-sectional area calculations, however, assume that the fiber bundles are of uniform diameter along their length. This was not necessarily the case. The fiber bundles were thinner at their origin and insertion than in the center. This should be considered for modeling procedures in the future.

We compared the mass measurements in this study to those of Faaborg-Andersen,[2] and the total masses of the human CT muscle were remarkably similar (942 mg, compared to 954 mg in his study). Cross-sectional area calculations were also similar (74 mm^2, compared to 60 mm^2) for the CT muscle. The masses of the TA muscle were not similar, because Faaborg-Andersen used the thyrovocalis portion only, while our study used the whole TA muscle (823 mg, compared to 318 mg).

Length. The length measurements in this study are within the range of length measurements reported by Hirano et al.[15] Their length measurements included both the membranous and cartilaginous portions. Their data suggested that the vocal fold length ranges from 17 to 21 mm in men, whereas in this study, the average length of the human TA muscle was 18.3 mm. The canine TA muscle in this study was 21.9 mm, which is longer than the 15 to 19 mm reported by Alipour-Haghighi and Titze.[16] The exterior portion of the CT muscle has longer muscle fiber bundles than the deeper portion of the muscle. Therefore, the difference in length measurements from those reported by Alipour-Haghighi and Titze is likely due to the fact that their samples were prepared from the medial portion of the canine TA muscle. Overall, the CT and TA muscles were significantly longer for our dogs than for our humans. The exception was the PO belly of the CT muscle. Although the difference was not statistically significant, the PO was longer in the human than in the dog.

Orientation and Pitch Control Issues. The differences between human and canine orientation angles of these muscles may be due to phylogenetic effects within the human race.[17,18] A more horizontal orientation of both of the CT bellies (PR and PO) may contribute to more backward displacement or retraction of the cricoid arch.[10,11] Many investigators[17-21] believe that this ventrodorsal sliding significantly affects changes in vocal fold length. It can be hypothesized that the human mechanism is more capable than the canine mechanism of producing dramatic changes in length. Perhaps that is why the human instrument is more versatile and can produce octave jumps in pitch within a second during singing. It may be noteworthy that the orientation of the TA muscle was not significantly different between the human and canine specimens.

Important Similarities and Differences. Pressman and Kelemen[22] stated that identifying certain details in the comparative anatomy of the larynx is of great importance to the physiologist, especially if laboratory experimentation is concerned. The greatest part of our scientific knowledge of the physiology of the larynx comes from observations of laboratory animals. In light of this fact, it is important to identify these differences in structure from one species to the next, even though the structures may appear very similar. Differences in innervation, quantitative muscle relationships, patterns of cartilaginous structure, and position and size are important. These differences contribute to the difficulty of applying knowledge gained by animal experimentation to human conditions.

The larynx of a large dog is similar to the human larynx in terms of size and vocal fold histology; however, dissection reveals differences in the CT and TA muscles that may be noteworthy to mention. An obvious difference was the absence of the vocal ligament in the canine. Titze et al[4] comment that the canine larynx is not an ideal model of the human larynx when discussing pitch control and vocal fold tissue morphology, because the vocal ligament is absent. On examination of both the human and canine cricoid cartilages and their relationship to the CT muscle, another obvious difference is noted. The human CT muscle appears to be set into a groove within the cricoid cartilage, and the canine CT muscle appears to be more exposed and bulky. Perhaps this could be related to body proportion differences within the two species. The neck of a dog is large and muscular in proportion to the rest of its body, while the neck of a human is proportionally much thinner. This could be related to each species' normal anatomic position and gravitational effects on that position. Perhaps it could also be related to the uniqueness of the human muscular framework that results in a more versatile and specialized instrument.

In spite of these differences, much of the information on the mechanics of vocal fold vibration has emerged from the study of excised canine larynges. A number of studies suggest that excised larynges do not accurately reproduce physiological conditions of vocal fold tension and mass during vibration.[23] Moore and Berke[8] believe that in vivo canine models appear to be better than excised larynges, because they maintain blood flow and intrinsic laryngeal muscular tension while preventing postmortem deterioration of the tissue. Choi et al also suggest that "despite differences in structure between human and canine larynges, in vivo canine studies are well suited for examining questions about fundamental frequency in phonation, because the basic gross function of major laryngeal muscles is quantitatively the same for the two species."[9(p774)] Hast[24] was a strong supporter of the use of the canine larynx and commented that the physiological similarity of the dog to the human supports the dog's value for research on the mechanisms of the larynx.

Moore and Berke recognized that "the use of animals to study laryngeal function provides a setting in which new concepts can be tested, while at the same time allowing manipulation of variables not easily controlled in humans."[8(p707)] Traditionally, investigators have used the dog as their principal animal model for laryngeal studies. That is why it is so important to know if the canine model truly represents the human larynx, when discussing pitch control.

CONCLUSION

Mass and cross-sectional area ratios of the human and canine specimens in this study suggest that the basic gross function of the CT and TA muscles is the same for the two species. However, the differences in fiber orientation could reflect different biomechanical functions in the two muscles. Data collected in this study from the CT muscle will be useful for calculating the torque acting on the CT joint to change vocal fold length. These calculations will help establish the functional relationship between the muscles in pitch control and provide a 3-dimensional picture of the dynamics of rotation. The data will also be used for a 3-dimensional reconstruction of the muscular attachments in the laryngeal framework. As always, it would be beneficial to have more subjects of different ages and sexes so that we could interpret differences in laryngeal control in the developmental and aging periods.

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REFERENCES

1. Titze IR. Phonation threshold pressure: a missing link in glottal aerodynamics. J Acoust Soc Am 1992;91:2926-35.
2. Faaborg-Andersen K. Electromyographic investigation of intrinsic laryngeal muscles in humans. Acta Physiol Scand Suppl 1957;41(suppl 140).
3. Hirano M, Vennard W, Ohala J. The function of laryngeal muscles in regulating fundamental frequency and intensity of phonation. J Speech Hear Res 1969;12:616-28.
4. Titze IR, Luschei ES, Hirano M. Role of the thyroarytenoid muscle in regulation of fundamental frequency. J Voice 1989;3:213-24.
5. Larson CR, Kempster GB. Voice fundamental frequency changes following discharge of laryngeal motor units. In: Titze IR, Scherer RC, eds. Vocal fold physiology: biomechanics, acoustics, and phonatory control. Denver, Colo: The Denver Center for the Performing Arts, 1983:91-104.
6. Hirano M. Morphological structure of the vocal cord as a vibrator and its variations. Folia Phoniatr (Basel) 1976;26:89-94.
7. Rubin H. Experimental studies on vocal pitch and intensity in phonation. Laryngoscope 1963;73:973-1015.
8. Moore DM, Berke GS. The effect of laryngeal nerve stimulation on phonation: a glottographic study using an in vivo canine model. J Acoust Soc Am 1988;83:705-15.
9. Choi H-S, Berke GS, Ye M, Kreiman J. Function of the thyroarytenoid muscle in a canine laryngeal model. Ann Otol Rhinol Laryngol 1993;102:769-76.
10. Arnold GE. Physiology and pathology of the cricothyroid muscle. Laryngoscope 1961;71:687-753.
11. Alipour-Haghighi F, Perlman AL, Titze IR. Tetanic response of the cricothyroid muscle. Ann Otol Rhinol Laryngol 1991;100:626-31.
12. Kempster G, Larson C, Kistler M. Effects of electrical stimulation of cricoarytenoid and thyroid muscles on fundamental frequency. J Voice 1988;2:221-9.
13. Negus BE. The comparative anatomy and physiology of the larynx. New York, NY: Grune & Stratton, 1949.
14. Siegel S. Nonparametric statistics for the behavioral sciences. New York, NY: McGraw-Hill, 1956.
15. Hirano M, Kurita S, Nagata K. Growth, development and aging of human vocal folds. In: Bless DM, Abbs JH, eds. Vocal fold physiology. San Diego, Calif: College-Hill Press, 1983:22-43.
16. Alipour-Haghighi F, Titze IR. Elastic models of vocal fold tissues. J Acoust Soc Am 1991;90:1326-31.
17. Sonninen A. The role of the external laryngeal muscles in length-adjustment of the vocal cords in singing. Acta Otolaryngol [Suppl] (Stockh) 1956(suppl 130).
18. Stone RE, Nuttall AL. Relative movements of the thy-

roid and cricoid cartilages assessed by neural stimulation in dogs. Acta Otolaryngol (Stockh) 1974;78:135-40.

19. Hollien H. In search of vocal frequency control mechanisms. In: Bless DM, Abbs JH, eds. Vocal fold physiology. San Diego, Calif: College-Hill Press, 1983:361-7.

20. Vilkman E. An apparatus for studying the role of the cricothyroid articulation in the voice production of excised human larynges. Folia Phoniatr (Basel) 1987;39:167-77.

21. Zaretsky LS, Sanders I. The three bellies of the canine cricothyroid muscle. Ann Otol Rhinol Laryngol Suppl 1992; 101(suppl 156).

22. Pressman J, Kelemen G. Physiology of the larynx. Arch Otolaryngol 1955;35:506-54.

23. Perlman AL, Titze IR, Cooper DS. Elasticity of canine vocal fold tissue. J Speech Hear Res 1984;27:212-9.

24. Hast MH. The primate larynx: a comparative physiological study of intrinsic muscles. Acta Otolaryngol (Stockh) 1969; 67:84-92.

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INTERNATIONAL CONFERENCE ON NEWBORN HEARING SCREENING DIAGNOSIS AND INTERVENTION

The International Conference on Newborn Hearing Screening Diagnosis and Intervention will be held in Milan, Italy, October 12-14, 2000. In Europe, contact Dr Ferdinando Grandori, Center of Biomedical Engineering, Polytechnic of Milan, piazza Leonardo da Vinci 32, I-20133 Milan, Italy; fax +39.02.2399.3360; e-mail NHS2000@biomed.polimi.it. In the United States, contact Dr Deborah Hayes, The Children's Hospital, 1056 E 19th Ave, B030, Denver, CO 80218; telephone (303) 861-6424; fax (303) 764-8220; e-mail: Hayes.deborah@ tchden.org.

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RADIATION-INDUCED HEARING IMPAIRMENT IN PATIENTS TREATED FOR MALIGNANT PAROTID TUMOR

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Radiation-induced hearing loss was evaluated in 21 patients with unilateral malignant parotid tumors treated with surgery and radiotherapy. The contralateral ear was used as a control. Eight patients (38%) were found to have a reduction in static compliance of the tympanic membrane (type B tympanogram) in the irradiated ear. By audiometry, significant hearing loss was found in 9 patients (43%). These hearing losses were mainly sensorineural, as shown by a similar reduction in both air and bone conduction, although mixed-type hearing loss existed in some patients. A statistically significant difference in incidence of 67% versus 0% ($p = .0085$) was noted for patients with a cochlear dose of greater than or equal to 60 Gy, in comparison to those receiving doses of less than 60 Gy. A type B tympanogram was also found to be a prognostic factor for significant sensorineural hearing loss. Patients with type B tympanograms had a much higher incidence of significant sensorineural hearing loss than those with type A tympanograms (88% versus 15%, $p = .02$). This study clearly shows that radiotherapy can induce significant hearing impairment, especially when the cochlear doses are higher than 60 Gy.

KEY WORDS — audiometry, hearing, parotid neoplasm, radiotherapy, tympanometry.

INTRODUCTION

Radiotherapy (RT) is a major treatment modality for many head and neck cancers. However, side effects on the statoacoustic organ may occur when the ear is included in the treatment field. Huang and Chu[1] reported that 44% of nasopharyngeal carcinoma (NPC) patients had ear trouble during and after RT. In the acute stage, patients often have the signs and symptoms of otitis media due to transient and reversible edema around the eustachian tube. Patients may also develop progressive and permanent hearing impairment in the chronic stage, which may be caused by direct damage to the ear structures.[2,3]

There is a wide range of incidence of radiation-induced permanent hearing impairment reported in the literature for malignancies arising in close proximity to the ears. In cases of NPC, the incidence varies from 3% to 54%.[1,4-6] In cases of parotid tumors, Evans at al[7] and Borsanyi and Blanchard[8] did not find any patient developing significant hearing impairment after RT. However, Schot et al,[9] Leach,[2] and Moretti[3] showed that hearing loss was very common following temporal bone irradiation, with an incidence rate as high as 50%. The discrepancy between these reports may be due to differences in radiation dose and techniques, definition of hearing impairment, or other factors. In the past, it was very difficult to spare the ear from the high–radiation dose region in these diseases for fear of compromising tumor control. With modern RT techniques, the radiation dose to the ear can be reduced with proper shielding. Therefore, it is of utmost importance to determine the threshold dose for the acoustic organ, which will offer us vital information in choosing the best RT technique.

In this retrospective study, patients with unilateral parotid gland tumors treated with surgery and irradiation were evaluated for possible hearing loss 2 to 14 years after RT. The difference in hearing between ears was evaluated, so that the contralateral ear was used as the control.

MATERIALS AND METHODS

From 1986 to 1992, 89 patients with unilateral parotid gland tumors were treated with curative intent in the Department of Radiation Oncology. Of those, 37 patients had died by the time of the study. Twenty-one of the 52 surviving patients agreed to receive a hearing examination. There were 15 female and 6 male patients, ranging in age from 19 to 82 years, with a median age of 49 years. Twenty patients received postoperative RT after parotidectomy, and 1 patient was treated with RT for recurrent tumor after surgery. The hearing evaluation was done 2 to 14

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TABLE 1. CALCULATED COCHLEAR DOSE TO IPSILATERAL AND CONTRALATERAL EARS

NTD (Gy)	*Ipsilateral*	*Contralateral*
≤20	0	12
20.1-30	0	4
30.1-40	0	2
40.1-46	0	3
46.1-55	3	0
55.1-60	3	0
60.1-65	9	0
≥65	6	0

NTD — normalized total dose calculated according to linear-quadratic model with α/β ratio of 3 Gy.

years after irradiation.

Fourteen patients were treated by the ipsilateral wedge pair technique. Of the 14 patients, 10 patients were treated with combined photon and electron beams and 4 patients were treated with photon beams only. The remaining 7 patients were treated with electron beams only. The target volume included the entire parotid region with an adequate safety margin. The treatment planning for each case was reviewed and doses to both ears were recalculated by a senior physicist. The total target dose ranged from 48 to 70 Gy, with a median dose of 60.8 Gy. The daily dose to the clinical target volume ranged from 1.8 to 2 Gy. The dose distribution to the irradiated and contralateral cochleas is shown in Table 1. Radiation was defined by biologically equivalent dose (normalized total dose; NTD) by the linear-quadratic model with an α/β ratio of 3 Gy for the ear and normalization of the total dose at 2 Gy per fraction.

Evaluation of hearing included an interview, a routine otorhinolaryngological examination, Amplaid type 455 tympanometry, pure tone audiometry with a Grason-Stadler GSI 33 audiometer, and measurement of speech reception threshold (SRT) and discrimination score. The possibility of tumor recurrence causing hearing loss was ruled out by detailed clinical examination and computed tomography (CT) if necessary.

Bone conduction at 250, 500, 1,000, 2,000, 4,000, and 8,000 Hz was used as an end point to estimate the degree of cochlear damage. Pure tone thresholds were evaluated in a soundproof room by standard criteria using masking when indicated, and equipment was calibrated to national standards. The SRT and speech discrimination score (SDS) were done to measure the ability of a patient to understand speech well above his or her hearing threshold. The Phonetically Balanced Maximal (P-B Max) scores of the SDS were usually tested at hearing levels 30 to 40 dB higher than the SRT. An objective hearing loss was defined as a mean difference in the hearing threshold between the irradiated and the contralateral ears of more than 15 dB in all frequency levels. Significant sensorineural hearing loss (SNHL) was defined as a 20-dB difference between the irradiated and contralateral ears for a minimum of 2 frequencies, as suggested by Anteunis et al.[10]

The correlation between the individual SNHLs observed with different parameters was tested by linear regression univariate analysis, with the significance level set at $p < .05$.

RESULTS

Subjective Evaluation. In the interview portion, no patients reported a significant difference in hearing between both ears before RT. Two patients had repeated otorrhea or infections at the lesion site preoperatively. One patient had had neurofibromatosis since childhood, with no evidence of acoustic neuroma noted in the preoperative CT scan. After completion of RT, 5 patients (23%) reported deterioration of hearing on the irradiated side. Two patients had several episodes of tinnitus on the irradiated side. One patient noticed contralateral tinnitus. Repeated external acoustic canal infections in the irradiated ear were found in 2 patients.

Otolaryngological Evaluation and Tympanometry. Otolaryngological examination revealed intact eardrums in both ears of all patients. The results of tympanometry were classified into types A, B, and C, as proposed by Hayes and Jerger.[11] On the irradiated ear, 8 patients (38%) had a type B tympanogram, which was most likely due to middle ear fluid accumulation. Actually, 6 of these 8 patients were found to have ear effusion during the otolaryngological checkup. In the rest, including the contralateral ears, a type A tympanogram, usually found in normal persons, was obtained.

Audiometry. Because middle ear effusion may induce conductive hearing loss, the impairment caused by cochlear damage needs to be further evaluated by calculating the difference between the bone conduction thresholds, masked if necessary, of the irradiated and contralateral ears. As shown in Fig 1, a marked hearing loss was found in the irradiated ears. Hearing loss of more than 15 dB for the irradiated ear was noted in 11 patients, 3 patients, 4 patients, 5 patients, 6 patients, and 10 patients for the respective frequency levels of 250, 500, 1,000, 2,000, 4,000, and 8,000 Hz. The mean hearing losses were 12.6 dB, 4 dB, 5.7 dB, 4.8 dB, 7.9 dB, and 11.2 dB, respectively. This result suggests that sensorineural damage is a common type of radiation-induced hear-

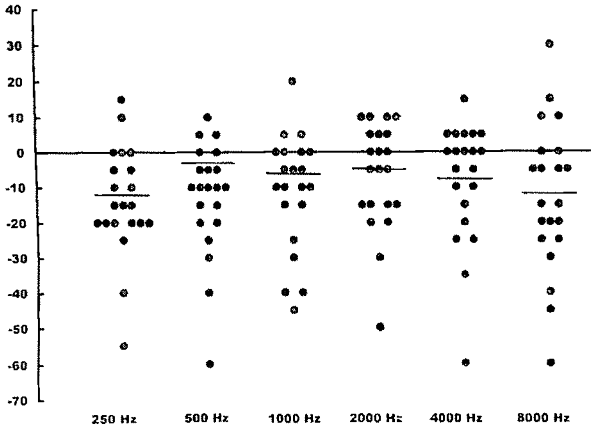
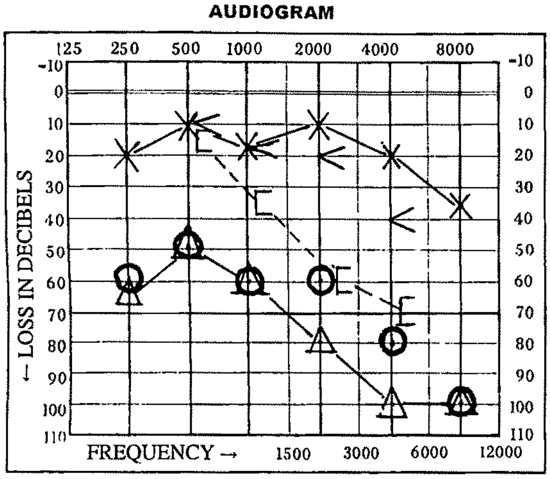


Fig 1. Audiometric results showing difference in decibels in bone conduction threshold between irradiated and nonirradiated ears at 250, 500, 1,000, 2,000, 4,000, and 8,000 Hz. Solid lines represent mean values for every frequency.

ing loss. However, in addition to the SNHL, a conductive deficit may contribute to the hearing loss. Five of the 8 patients with type B tympanograms had mixed-type hearing loss instead of pure SNHL, illustrated by the representative pure tone audiogram in 1 patient shown in Fig 2.



SPEECH HEARING TESTS

	Right	Left
SRT	55 dB	10 dB
PB score	76% (at 95 dB; 65 dB mask for left ear)	100% (at 50 dB; 0 dB mask for right ear)

Fig 2. Audiogram showing significant unilateral mixed-type (predominantly sensorineural) hearing loss in 54-year-old female patient after radiotherapy for right parotid tumor (estimated right and left cochlear doses are 7,824 and 1,064 cGy, respectively). O — Air conduction of right ear, unmasked; Δ — air conduction of right ear, masked; < — bone conduction of right ear, unmasked; [— bone conduction of right ear, masked; × — air conduction of left ear, unmasked; SRT — speech reception threshold; PB — Phonetically Balanced.

TABLE 2. ASSOCIATION BETWEEN SUBJECTIVE (CLINICAL) AND OBJECTIVE (AUDIOMETRIC) HEARING LOSS

*Objective Hearing Loss**	*Subjective Hearing Loss*	*p†*
250 Hz		
Yes	33% (4/12)	.338
No	11% (1/9)	
500 Hz		
Yes	63% (5/8)	.0275
No	0% (0/13)	
1,000 Hz		
Yes	71% (5/7)	.001
No	0% (0/14)	
2,000 Hz		
Yes	50% (4/8)	.047
No	8% (1/13)	
4,000 Hz		
Yes	17% (1/6)	1.000
No	27% (4/15)	
8,000 Hz		
Yes	20% (2/10)	1.000
No	27% (3/11)	

*Defined as 15-dB loss of hearing threshold between irradiated and control ears.

†Fisher's exact test (2-tailed).

A subjective hearing loss is defined as any degree of hearing impairment reported by the patients. An objective hearing loss is defined as a mean difference in the hearing threshold between the irradiated and the contralateral ears of more than 15 dB in all frequency levels. The correlation between subjective (clinical) and objective (audiometric) hearing loss was evaluated. A significant association was found only at 500, 1,000, and 2,000 Hz, which is the frequency range for audible speech (Table 2). Significant SNHL was found in 9 patients. The incidence of subjective hearing loss (5/21 or 24%) is much lower than the incidence of objective significant SNHL (9/21 or 43%), so radiation-induced hearing loss might be subclinical.

The SRTs and SDSs were in accordance with the pure tone audiograms. The SRT results were almost equal to the hearing thresholds at 500, 1,000, and 2,000 Hz. The SRTs for patients with significant SNHL and without SNHL ranged from 15 to 95 dB (median 35 dB) and 0 to 40 dB (median 15 dB), respectively. Normal tolerances for SDSs were set ranging from 88% to 100% as previously defined in the literature.[12] A P-B Max score of less than 88% in the irradiated ears was found in 3 of 9 patients with significant SNHL. All 3 patients with abnormal P-B Max scores had hearing thresholds of more than 40 dB in the audible speech levels (500 to 2,000 Hz). The remaining 6 patients with significant SNHL but nor-

TABLE 3. RISK FACTOR ANALYSIS FOR RADIATION-INDUCED HEARING LOSS

Risk Factor	*Patients With Hearing Loss**	*p*
Dose (to cochlea)		
≥60 Gy	60% (9/15)	.0085
<60 Gy	0% (0/6)	
Age		
≥50 y	67% (8/12)	.08
<50 y	11% (1/9)	
Type B tympanogram		
Yes	88% (7/8)	.02
No	15% (2/13)	

*Defined as 20-dB loss of hearing threshold between irradiated and control ears for at least 2 frequencies.

TABLE 4. CORRELATION BETWEEN RADIATION DOSE AND SIGNIFICANT SNHL, TYPE B TYMPANOGRAM, AND SUBJECTIVE HEARING LOSS

Radiation Dose to Cochlea	*Significant SNHL*	*Type B Tympanogram*	*Subjective Hearing Loss*
≥6,000 cGy	9/15 (60%)	8/15 (53%)	5/15 (33%)
<6,000 cGy	0/6 (0%)	0/6 (0%)	0/6 (0%)

SNHL — sensorineural hearing loss.

mal P-B Max scores had either a hearing threshold of less than 40 dB or a hearing loss mainly in the higher-frequency regions (4,000 to 8,000 Hz). Under both conditions, P-B Max scores may not change. This finding suggests that the significant hearing loss in the audible range of the irradiated ears was caused by neural damage. With regard to the 12 patients without significant SNHL, only 1 patient had similar abnormal P-B Max scores in the irradiated and contralateral ears, suggesting presbycusis.

The effect of dose and other possible factors causing significant SNHL was further evaluated. As shown in Table 3, the radiation dose and development of a type B tympanogram were significantly related to SNHL. Nine (60%) of the 15 patients with a cochlear dose higher than 60 Gy developed clinically relevant hearing loss, with no hearing impairment noted for patients receiving doses less than 60 Gy ($p < .01$). Seven of 8 (88%) patients with type B tympanograms had significant SNHL, but only 2 of the 13 patients (15%) with type A tympanograms developed SNHL ($p < .05$). The effect of age at the time of examination on SNHL cannot be fully evaluated due to the limited case number, although its p value reached borderline significance ($p = .08$). The median follow-up for patients with significant SNHL was 7.22 years as compared to 5.78 years for patients without SNHL, with no statistically significant difference ($p = .41$). The follow-up time is not a prognostic factor for hearing loss. As shown in Table 4, there was a strong correlation between the radiation dose and the development of a type B tympanogram and SNHL. All patients developing type B tympanograms or subjective hearing loss had a cochlear dose greater than or equal to 6,000 cGy. It is possible that the association between the development of a type B tympanogram and SNHL is due to a higher radiation dose. We concluded that radiation dose is the most important factor in sensorineural, conductive, and subjective hearing loss.

DISCUSSION

In this study, we investigated the radiation-induced hearing impairment of patients with unilateral malignant parotid tumors by using the contralateral ear as an internal control. This study is flawed because of its retrospective nature and the unavailability of a pre-RT baseline value. However, results from other studies showing that surgery of parotid tumors did not affect hearing function[13] and that the contralateral ear could be used as an internal control[10] support the conclusion that our study is valuable and could be used to answer the questions we tried to address.

In the tympanometry study, all type B tympanograms occurred in irradiated ears. This could be partially explained by serous effusion in the middle ear and disturbance of ventilation and drainage caused by edema of the mucous membrane, which leads to obstruction of the eustachian tube.[14] In an animal study, Ohashi et al[15] noted serous effusions in the tympanic cavities of guinea pigs irradiated with a single dose of 30 Gy. They also noted degeneration of cilia or ciliated cells and capillary injury with increased permeability. Berg and Lindgren[16] also found that fibrosis in the walls of the middle ear and a thickened, fibrotic mucous membrane developed in the ears of rabbits 52 weeks after they received a high radiation dose of 30 Gy in a single fraction or a total dose of 105 Gy in a fraction size of 3.5 Gy. In human studies, persistent otitis media develops, with a low but regular incidence varying between 2% and 14%.[4-6] Huang and Chu[1] reported that 44% of 1,032 NPC patients treated for 7 to 8 weeks with bilateral opposing fields of 4,600 cGy and anterior oblique fields at another 2,400 cGy to the nasopharynx developed postirradiation ear trouble due to eustachian tube obstruction. In our study, 8 of 21 patients developed a type B tympanogram. Five of them had mixed-type hearing loss and 6 had ear effusion at the otolaryngological checkup, suggesting that conductive hearing loss may also contribute to the radiation-induced hearing impairment. In a prospective study, Kwong et al[17] showed that among NPC patients, a high incidence of SNHL was found in patients with postirradiation serous otitis media (46.9%). Further multivariate analysis confirmed that postirradiation serous otitis media was a significant prognostic factor for persistent SNHL. In our study, we also found a similar association between patients receiving doses above 60 Gy and a type B tympanogram and significant SNHL. An association between chronic otitis media (COM) and SNHL is controversial in the literature. Paparella and Brady[18] reported absolute bone conduction losses between 18 and 33 dB across the frequency range of 500, 1,000, 2,000, and 4,000 Hz in patients with COM. They explained that insidious SNHL may be caused by trans–round window biochemical alteration of first the perilymph and then the endolymph, resulting in gradual end organ dysfunction.[18] However, other investigators indicate that the association of SNHL with COM is less significant, ranging from −0.5 to 12.8 dB across the frequencies.[19,20] In our study, we defined significant SNHL as a difference of 20 dB or more for at least 2 frequencies between irradiated and contralateral ears. We cannot prove whether a type B tympanogram or long-term ear effusion contributed to SNHL or whether they were just another manifestation of individual sensitivity to radiation. However, the middle ear problem usually occurs before permanent sensorineural damage and can be used as a prognosticator for subsequent SNHL.

Concerning radiation damage to the cochlea, much histologic evidence has been brought out in the literature. In the study of Gamble et al,[21] animals were irradiated with doses of 5, 10, 20, 30, 40, 50, or 60 Gy. The earliest changes were seen in the stria vascularis with a dose level above 30 Gy. As the dose increased to 50 to 60 Gy, changes in hair cells with cell boundary dissolution and elevation of the tectorial membrane were found. Atrophy of the stria vascularis, spiral ligament, and basilar membrane, necrosis of the organ of Corti, and an absence of neural elements were also seen in human studies.[22,23] Bohne et al[24] reported that the delayed effects of radiation in the ears of chinchillas consisted of degeneration of sensory and supporting cells and loss of 8 nerve fibers in the organ of Corti. In our study, we used the bone conduction threshold at each frequency level to evaluate SNHL. The incidence varies from series to series. Anteunis et al[10] reported in a prospective study that up to 50% of the patients (9 of 18) developed both clinically relevant conductive loss and SNHL in the irradiated ear. The contralateral ear remained unaltered. They suggested that a total dose of 50 Gy, with a daily fraction of 2 to 2.5 Gy, might induce significant changes in the inner ear as well as in the auditory nerve and auditory brain stem. Grau et al[25] found a significantly higher incidence of SNHL with doses above 50 Gy. In our study, the incidence of significant objective unilateral hearing impairment is 42.8% (9/21). Significant hearing impairment was only seen in those patients whose cochlear doses were higher than or equal to 60 Gy, and this finding suggests that doses of less than 60 Gy delivered by conventional daily fractionation are relatively safe. This safe dose range is higher than that in some reports, but similar to that suggested by Schot et al,[9] who found that a dose to the cochlea of less than 55 Gy seldom caused hearing loss, in contrast to doses exceeding 65 Gy. According to our results, the tolerance dose of the inner ear should be between 5,000 and 6,000 cGy. The "TD5/5," denoting a 5% complication probability over 5 years, for SNHL set at 60 Gy by Emami et al[26] is in agreement with our findings. The SRT and P-B Max scores further support the belief that the damage to hearing was caused by neural dysfunction.

We conclude that RT can induce significant hearing loss in the irradiated ear, especially when the cochlear dose is higher than 60 Gy. In application to clinical practice, we will use 60 Gy as the tolerance dose for the inner ear in 3-dimensional computer-aided treatment planning for various head and neck tumors.

REFERENCES

1. Huang SC, Chu GL. Nasopharyngeal cancer: study II. Int J Radiat Oncol Biol Phys 1981;7:713-6.

2. Leach W. Irradiation of the ear. J Laryngol Otol 1965; 79:870-80.

3. Moretti JA. Sensorineural hearing loss following radiotherapy to the nasopharynx. Laryngoscope 1976;86:598-602.

4. Baker SR. Nasopharyngeal carcinoma: clinical course and results of therapy. Head Neck Surg 1980;3:8-14.

5. Urdaneta N, Fischer JJ, Vera R, Gutierrez E. Cancer of the nasopharynx: a review of 43 cases treated with supervoltage radiation therapy. Cancer 1976;37:1707-12.

6. Lee AWM, Law SCK, Ng SH, et al. Retrospective analysis of nasopharyngeal carcinoma treated during 1976-1985: late complication following megavoltage irradiation. Br J Radiol 1992;65:918-28.

7. Evans RA, Liu KC, Azhar T, Symonds RP. Assessment of permanent hearing impairment following radical megavoltage radiotherapy. J Laryngol Otol 1988;102:588-9.

8. Borsanyi S, Blanchard CL. Ionizing radiation and the ear. JAMA 1962;181:958-61.

9. Schot LJ, Hilgers FJM, Keus RB, Schouwenburg PF, Dreschler WA. Late effects of radiotherapy on hearing. Eur Arch Otorhinolaryngol 1992;249:305-8.

10. Anteunis LJC, Hendriks JJT, Manni JJ. A prospective longitudinal study on radiation-induced hearing loss. Am J Surg 1994;168:408-11.

11. Hayes D, Jerger J. Impedance audiometry in otologic diagnosis. Otolaryngol Clin North Am 1978;11:759-67.

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PROGRESSIVE SENSORINEURAL HEARING IMPAIRMENT IN PROFESSIONAL FISHERY DIVERS

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There have been many reports of a high rate of hearing impairment in divers. A prospective study was performed to determine whether sensorineural hearing acuity in the same divers deteriorated faster than in a normal population as they continued diving. After an observation period of approximately 5 years, audiometric examination was performed on a group of professional fishery divers who had normal hearing or sensorineural hearing loss at the time of initial study. Thirty-three ears of 18 divers were included in statistical analyses. The average hearing deterioration in the divers in 5 years, after elimination of the aging effect, was 6.6 dB (SD 4.5) and was statistically significant. We concluded that the hearing acuity of our subjects deteriorated faster than that of normal Japanese.

KEY WORDS — diving, hearing, hearing impairment.

INTRODUCTION

Whether diving has a harmful effect on hearing is controversial. Some authors have indicated that diving per se does not result in auditory impairment. Shilling and Everley[1] concluded from their study of submarine personnel and divers that diving did not cause hearing losses unless associated with aero–otitis media. Brady et al[2] performed an audiometric survey of navy divers and found no significant difference in results compared to those of a normal population of nondivers. Brink[3] also detected no significant differences in hearing sensitivity between navy divers and nondiving navy men.

On the other hand, some investigators have described a high rate of hearing impairment in divers. Borasi and Sperati[4] found hearing loss in 56% of 108 professional divers, and stated that the percentage of hypoacusis is bound to the time the subaqueous activity was continued. Zannini et al[5] examined the hearing acuity of 123 professional divers and found that 76.9% of them had impaired hearing, a percentage significantly higher than that for comparable nondivers. Dembert et al[6] reported that the average auditory acuity of navy divers (mean age 28.3 years) is comparable to that observed in the general population at age 50 to 59 years. Edmonds[7] surveyed professional abalone divers and found that over 60% had unacceptable sensorineural, high-frequency deafness.

We performed an audiometric survey of a group of 31 professional fishery divers from Kohzu Island of Japan in March 1989. The results of this investigation have been reported elsewhere (Ohgaki et al,[8] 1992). Briefly, we found that 71% of ears (44 of 62 ears) had sensorineural hearing loss, and of these, 27.2% exhibited low-frequency loss, 31.8% high-frequency loss, and 41.0% high- and low-frequency loss. In addition, it appeared that the severity of hearing loss was related to the number of years of diving. There was only 1 ear in 1 subject with a past history of acute sensorineural hearing loss thought to be caused by inner ear barotrauma, and in all other cases, sensorineural hearing loss appeared to progress chronically with continuation of diving.

Although we found that the rate of sensorineural hearing loss was quite high among our subjects, it remained uncertain whether their sensorineural hearing impairment was actually progressing with time. The purpose of the present study was to determine whether sensorineural hearing acuity in the same divers deteriorated faster than that in a normal population as they continued diving. We performed this prospective study after an observation period of approximately 5 years.

MATERIALS AND METHODS

Subjects. Kohzu Island is a small Pacific island in Japan with a population of approximately 2,400. Professional fishery divers on the island perform fishing almost every day, except in winter or during bad weather. In summer, they engage in traditional *oiko-*

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mi fishing, in which the divers drive fish into a fixed net, while wearing diving gear with compressed air hoses. In spring and fall, they skin-dive to obtain lobsters and seaweed. Diving is performed mainly at 12 to 18 m of depth, and sometimes at 20 to 30 m.

Of the 31 fishery divers that were studied in March 1989, 19 were reexamined at Kohzu Island National Health Insurance Clinic in November 1993. All were male. Of those who were reexamined, all but 1 had normal hearing or sensorineural hearing loss at the time of the initial examination. The 1 patient who had exhibited mixed hearing impairment due to repeated otitis media was excluded from this study, since our aim was to examine how diving affects the sensorineural hearing system. The mean age of the remaining 18 subjects was 41.4 years (SD 11.9), and their diving experience ranged from 6 to 45 years (mean 22.6, SD 13.9) at the time of reexamination.

Methods. As in the initial examination, previous ear disease was questioned for in detail, especially to confirm the absence of inner ear barotrauma and decompression sickness during and after diving. In addition, we confirmed the absence of other diseases associated with sensorineural hearing loss such as hematologic disease, hypertension, diabetes, syphilis, etc. One of the authors, who had performed the same examination in the initial study, checked the ears using an otoscope and measured the audiometric acuity of all subjects.

Since there is no soundproof room on Kohzu Island, the hearing acuity of the subjects was tested in a room in the clinic that was the same room used for the initial study. Although the background noise was not checked, we believe our data are reliable, since the room was very quiet and the hearing acuities of the authors measured in the room were almost the same as those measured in the soundproof room of our hospital. The audiometer used (AA-63C, RION, Tokyo, Japan) was also the same one used for the initial examination, and was calibrated according to International Standards Organization standards. Air conduction thresholds were determined for the frequencies 125, 250, 500, 1,000, 2,000, 4,000, and 8,000 Hz. Bone conduction thresholds were measured only when the air conduction thresholds exceeded 20 dB, to ensure that hearing loss was sensorineural. In air conduction measurements, masking for the lower-threshold ear was performed when the difference between ears was 40 dB or more. In bone conduction tests, masking for the contralateral ear was routinely performed. If the hearing loss exceeded the maximum output of the audiometer, the value of hearing loss was defined as maximum output +5 dB.

Statistical Analyses. To represent the hearing acuity of each ear, we defined "average hearing level" as (a + 2b + 2c + d)/6, where a, b, c, and d are the hearing thresholds at 500, 1,000, 2,000, and 4,000 Hz, respectively. We calculated the value for each ear in the former and the latter years to determine how hearing acuity had changed over 5 years.

Since age greatly affects hearing acuity, elimination of this effect must be performed prior to evaluation of results. We subtracted the hearing level of normal subjects at the corresponding age from the measured hearing level of our subjects to eliminate the effect of aging on hearing acuity. The resulting value was hypothesized to be the hearing loss caused by diving. The mean hearing thresholds of otologically healthy Japanese men not working in noisy circumstances were reported by Okamoto.[9] In his study, he examined a large number of normal subjects and determined mean hearing thresholds for each frequency (250, 500, 1,000, 2,000, 4,000, 8,000 Hz) in different age groups. Using his data, we calculated the average hearing levels of normal subjects at 25, 33, 38, 43, 48, 53, 58, and 63 years of age. To determine mean hearing thresholds for subjects whose ages were between those given in his report, we used interpolation assuming that hearing change by age is linear. Since Okamoto's report did not include mean hearing thresholds for those less than 25 years old, we used the thresholds at 25 years for subjects under 25 years.

To determine which frequencies were most affected, we used a similar method. First, we subtracted the mean hearing levels of normal subjects at the corresponding age from the measured hearing levels of our subjects by frequency (250, 500, 1,000, 2,000, 4,000, 8,000 Hz). The resulting values were hypothesized to be the diving-related hearing losses at each frequency at a certain age. Next, we calculated the mean ± SD of these values in 1989 and 1993 separately by frequency. Finally, we calculated the mean ± SD of the 5-year difference in the values for the identical ears by frequency, which we hypothesized to be the age-adjusted hearing deterioration caused by diving in 5 years.

The paired Wilcoxon test and Wilcoxon-Mann-Whitney test were used to determine the significance of differences.

RESULTS

Of the 36 ears of the 18 subjects, 3 were excluded from this study: 1 because of acute otitis media, 1 for a perforated tympanic membrane, and 1 for myringitis. Therefore, 33 ears (16 left, 17 right) in 18 subjects were included for statistical analyses. Air-bone gaps were negligible in all these ears.

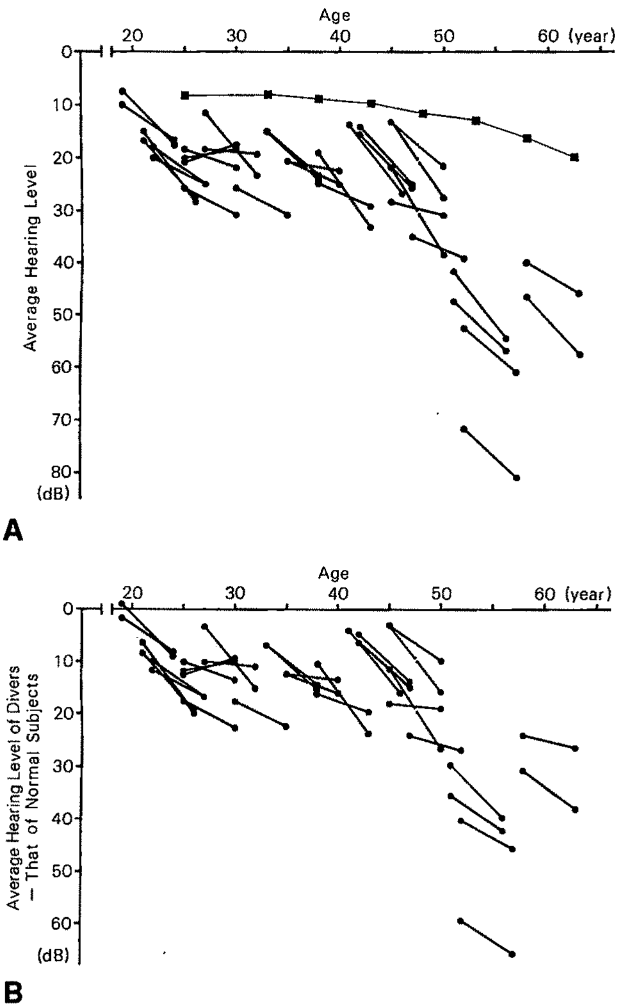

Fig 1. A) Average hearing levels of divers (33 ears) in March 1989 and November 1993 (closed circles connected by straight line) and that of normal Japanese men (data from Okamoto[9]; closed squares). B) Results of subtraction of average hearing levels of normal Japanese men at corresponding ages from those of divers in March 1989 and November 1993.

The average hearing levels of the 33 ears in the initial and most recent examinations are plotted in Fig 1A, along with those of normal Japanese given in Okamoto's[9] report. Figure 1B shows the values obtained by subtracting average hearing levels of normal subjects at corresponding ages from those of the divers. The results clearly indicate that the hearing acuity of our subjects tended to deteriorate faster than that of controls. The average hearing deterioration in divers in 5 years after elimination of the aging effect was 6.6 dB (SD 4.5), and the deterioration was significant ($p < .0001$, paired Wilcoxon test). On examination of age-adjusted hearing deterioration by frequency, 1,000 Hz was the most affected, followed by 500 Hz, and the hearing deteriorations at 500, 1,000, and 2,000 Hz were significant (see Table).

RESULTS OF SUBTRACTION OF MEAN HEARING THRESHOLD OF NORMAL SUBJECTS AT CORRESPONDING AGES FROM THAT OF DIVERS IN MAY 1989 AND NOVEMBER 1993, AND AGE-ADJUSTED HEARING DETERIORATION IN 5 YEARS (MEAN ± SD)

Frequency (Hz)	*Result of Subtraction of Mean Hearing Threshold of Normal Subjects From That of Divers (dB)*		*Age-Adjusted Hearing Deterioration in 5 y (dB)*
	In 1989	*In 1993*	
250	19.4 ± 9.9	22.4 ± 10.3	3.0 ± 9.9 (p = .39)
500	16.3 ± 8.6	22.9 ± 9.4	6.6 ± 10.0 (p = .0045)
1,000	8.6 ± 5.5	19.3 ± 6.8	10.7 ± 6.2 (p < .0001)
2,000	15.4 ± 21.4	19.3 ± 21.0	3.9 ± 6.0 (p = .0015)
4,000	22.5 ± 25.6	26.0 ± 21.6	3.5 ± 9.7 (p = .063)
8,000	24.8 ± 25.9	28.5 ± 28.0	3.8 ± 12.2 (p = .25)

To compare young and old subjects, we divided our subjects into 2 groups: those not exceeding 40 years and those over 40 years of age at the time of reexamination. The average hearing deterioration after eliminating the aging effect was 5.5 dB (SD 4.6) in the former group and 7.6 dB (SD 4.2) in the latter. Although the deterioration was greater in the group of older subjects, the difference was not significant ($p = .25$, Wilcoxon-Mann-Whitney test).

The audiograms of 3 representative cases for March 1989 and November 1993 are shown in Fig 2.

DISCUSSION

As noted in the Introduction, there have been many studies of hearing impairment in divers. However, most have not been prospective, but rather, cross-sectional studies. To the best of our knowledge, only 1 study of this type of hearing impairment using a prospective method is available. This study, performed by Molvær and Albrektsen,[10] was conducted on 116 professional divers with an observation period of approximately 6 years. They concluded that divers' hearing deteriorated faster than that of unscreened nondivers, consistent with our findings. Molvær and Lehmann[11] stated that their subjects included various kinds of divers, and that many of them were exposed to high noise levels. Our subjects, on the other hand, are more homogeneous and had had less noise exposure, similar to those studied by Edmonds.[7] Our subjects' diving schedules were comparatively similar, although there were some differences from person to person.

Our results clearly show that the hearing of divers deteriorated faster than that of normal subjects, con-

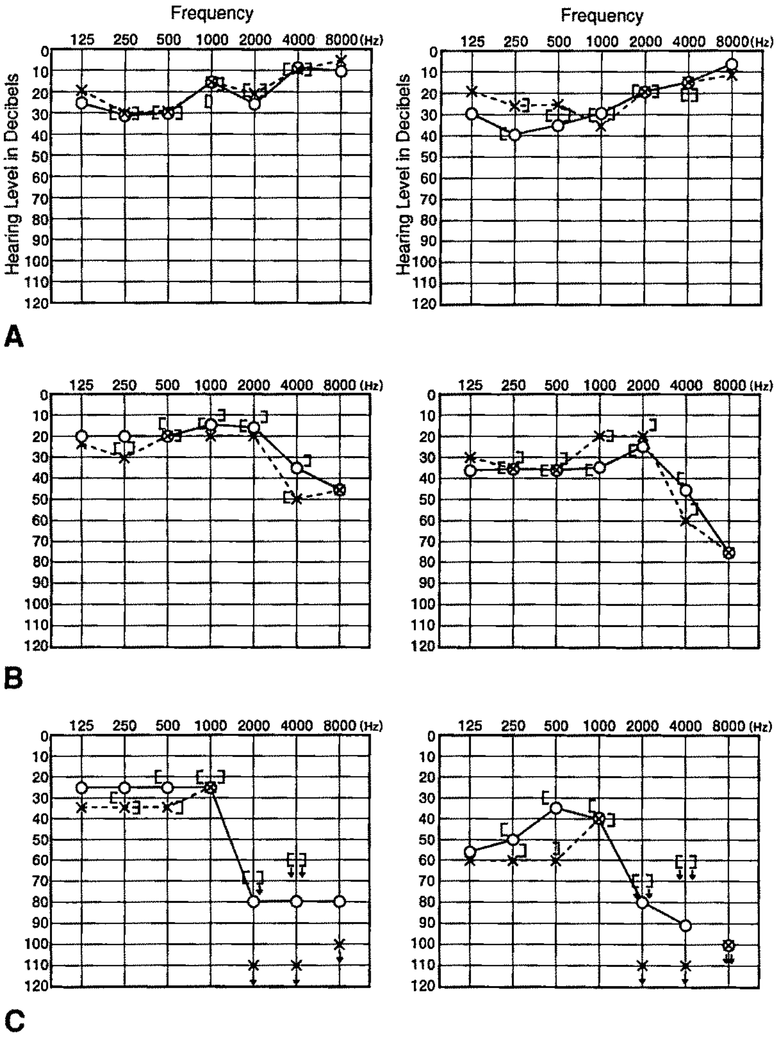

Fig 2. Audiograms of representative cases. O and × indicate right and left ear air conduction scores, respectively; [and] represent right and left ear bone conduction scores, respectively. Left audiograms were obtained in March 1989, and right audiograms in November 1993. A) 27-year-old diver with 6 years of diving by 1993. B) 43-year-old diver with 28 years of diving by 1993. C) 57-year-old diver with 43 years of diving by 1993.

sistent with the findings of Molvær and Albrektsen.[10] They made statistical analyses hypothesizing that divers' hearing would deteriorate linearly with amount of diving experience. However, our results indicate that the time course of hearing deterioration is more complex than they assumed. The amount of deterioration we detected differed considerably from person to person (Fig 1) and sometimes from left to right ears in an individual (see Fig 2B as an example) during the same period.

Most previous investigators found that greater hearing deterioration in divers occurred at high frequencies. In our subjects, however, age-adjusted hearing deterioration in 5 years was significant only at 500, 1,000, and 2,000 Hz. There appear to be 2 reasons for this. First, diving methods, schedules, and noise circumstances during diving differ between the subjects in previous studies and ours. In particular, previous studies tended to include subjects who were exposed to high noise levels during diving. In these circumstances, the influence of acoustic trauma, which often causes high-frequency sensorineural hearing loss, cannot be ruled out. Second, our study was an age-adjusted prospective study, while most of the previous studies were not. Since age greatly affects hearing acuity, particularly at high frequencies, previous studies may have confused the effects of aging and diving on hearing.

The cause of hearing deterioration in our subjects is unclear. Decompression sickness seems unlikely, since there was no history of acute hearing loss or decompression sickness in any of the organ systems in our subjects. Clinical inner ear barotrauma may have contributed to the hearing deterioration to some extent, since a few divers mentioned having experienced the sensation of ear squeezing while diving. Nevertheless, it seems unlikely that clinical inner ear barotrauma was the main cause of hearing deterioration, since most of the divers did not experience this sensation, and even those who did did not notice any hearing loss after it.

It is possible that repeated compression and decompression was the cause of hearing deterioration in our subjects. Zannini et al[5] mentioned that repeated minor unnoticed barotrauma may have the greatest importance in the genesis of hearing loss in divers. In an animal study, Wilkes et al[12] showed, using minipigs, that cochlear degeneration, particularly loss of hair cells, was observed after a degree of repeated compression-decompression exposure that is generally thought to be safe for humans. Since human divers make efforts to equalize middle ear pressure during dives, whereas minipigs presumably do not, as stated in the report of Wilkes et al, their results cannot be applied in any simple fashion to determination of the cause of hearing impairment in divers. Nevertheless, their results suggest that minute hair cell damage might occur with repeated, long-term compression-decompression cycles in professional divers.

REFERENCES

1. Shilling CW, Everley IA. Auditory acuity in submarine personnel. US Navy Med Bull 1942;40:664-86.

2. Brady JI Jr, Summitt JK, Berghage TE. An audiometric survey of Navy divers. Undersea Biomed Res 1976;3:41-7.

3. Brink GJ. Een Onderzoek naar gehoorverlies bij duikers van de Koninklijke Marine. Ned Milit Geneesk T 1977;30:55-61.

4. Borasi G, Sperati G. Il danno uditivo nei sommozzatori. Ann Laringol Otol Rinol Faringol 1968;67:36-43.

5. Zannini D, Odaglia G, Sperati G. Auditory changes in professional divers. In: Lambertsen CJ, ed. Underwater physiology V. Proceedings of the Fifth Symposium on Underwater Physiology. Bethesda, Md: Federation of American Societies for Experimental Biology, 1976:675-84.

6. Dembert ML, Mooney LW, Ostfeld AM, Lacroix PG. Multiphasic health profiles of navy divers. Undersea Biomed Res 1983;10:45-61.

7. Edmonds C. Hearing loss with frequent diving (deaf divers). Undersea Biomed Res 1985;12:315-9.

8. Ohgaki T, Nigauri T, Okubo J, Komatsuzaki A. Exostosis of the external auditory canal and sensorineural hearing loss in professional divers [in Japanese]. J Otolaryngol Jpn 1992;95:1323-31.

9. Okamoto M. Hearing condition and mean hearing level by age. In: Shidara T, ed. Otolaryngological features in old age [in Japanese]. Tokyo, Japan: Kanehara Publications, 1989:11-8. (Jibiinkouka-Toukeibugeka MOOK; vol 12).

10. Molvær OI, Albrektsen G. Hearing deterioration in professional divers: an epidemiologic study. Undersea Biomed Res 1990;17:231-46.

11. Molvær OI, Lehmann EH. Hearing acuity in professional divers. Undersea Biomed Res 1985;12:333-49.

12. Wilkes MK, Palmer AC, Pearce PC, Luff NP, Halsey MJ, Calder IM. Cochlear degeneration in minipigs after repeated hyperbaric exposures. Undersea Biomed Res 1989;16:139-52.

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SPONTANEOUS VERTEBRAL ARTERY DISSECTION MIMICKING ACUTE VERTIGO

CASE REPORT

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A patient with acute vertigo, and normal findings on neurologic examination, was found to have vertebral artery dissection (VAD). This case shows that the clinical picture of VAD can mimic vertigo of labyrinthine (ie, peripheral) origin.

KEY WORDS — chiropractic manipulation, vertebral artery dissection, vertebrobasilar system, vertigo.

INTRODUCTION

Vertebral artery dissection (VAD) is an uncommon disorder in which blood finds its way into the wall of the artery, probably through a tear in the endothelial surface, causing brain ischemia or subarachnoid hemorrhage.[1] In most patients, symptoms and signs develop gradually within hours to days. These include prominent headache, typically unilateral, as well as localized upper cervical pain. Features of lateral medullary syndrome are usually present, with symptomatic vertigo, ataxia and falling toward the side of the lesion, loss of the sense of pain and temperature sensation of the ipsilateral face and contralateral body, dysphagia with ipsilateral palate and vocal cord paralysis, and ipsilateral Horner's syndrome.[2] Mokri et al[3] found that in 25 patients with spontaneous VAD, the initial manifestation was pain in 60% (headache or neck pain), focal neurologic deficit in 20%, and vertigo and oscillopsia in 20% of cases. These initial symptoms usually stabilize in 1 to 2 weeks, leaving fluctuating ischemic symptoms that last for months. Repeated arteriographic studies have shown that at least 85% of cases resolve, or substantially improve, within 2 to 3 months.[3,4]

Vertebral artery dissection is the most common cause of lateral medullary syndrome in young adults. The mean age is about 40 years. The mechanism of injury is usually neck torsion, but the precise pathogenesis is unclear, and may include frequent or sudden neck movement, trauma, chiropractic manipulation, intubation, and swimming.[5-10] Cervical rotation and extension can compress the carotid and vertebral arteries against bony structures, possibly precipitating dissection in predisposed subjects.[4]

The diagnosis of VAD is confirmed by the characteristic angiographic findings, which include scalloped, irregular narrowing (with or without a pseudoaneurysm) and a double lumen.

We present an unusual case of VAD mimicking an acute attack of peripheral vertigo.

CASE REPORT

A 35-year-old, healthy man was admitted to the emergency room of the Hadassah University Hospital with acute, severe vertigo accompanied by nausea, vomiting, and unsteadiness that had lasted for a few hours. The vertigo started while he was repairing an industrial air conditioning system. There were no other complaints such as headache or neck pain. On admission he appeared pale, with vomiting. A spontaneous third-degree, right-beating nystagmus with a rotatory component was observed. The findings of the rest of the physical and otolaryngological examination were within normal limits. A full neurologic and neuro-ophthalmologic examination showed no evidence of a focal neurologic deficit, and the diagnostic impression was one of peripheral vertigo

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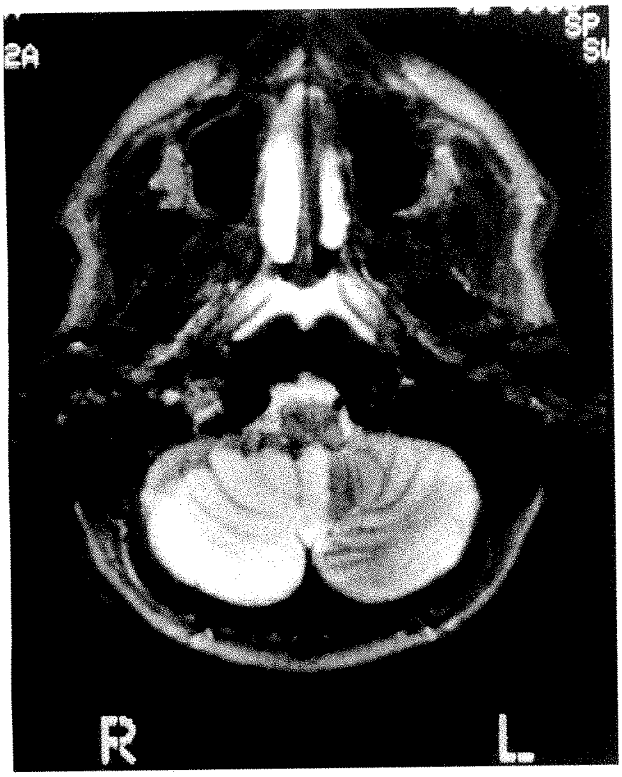

Fig 1. Axial T2-weighted magnetic resonance image shows acute large right cerebellar infarction in distribution of right posterior inferior cerebellar artery.

(ie, labyrinthine dysfunction). The audiological evaluation consisted of pure tone audiometry, speech reception threshold, speech discrimination, tympanometry, acoustic reflex, and auditory brain stem response (ABR) measurements, all of which were completely normal.

Computed tomography (CT) was performed, as is the protocol at our hospital for patients presenting with acute, severe vertigo. An infused CT scan of the brain showed asymmetric dilatation of the ventricular system. Therefore, magnetic resonance imaging (MRI) of the head showed aqueductal narrowing. Acute right cerebellar infarction in the posterior inferior cerebellar artery territory was also seen (Fig 1), which occupied the lower part of the cerebellum including the caudal vermis, yet the brain stem was intact. Angiography of the carotid and vertebral arteries demonstrated severe stenosis of the right vertebral artery at the C6-7 level, which was compatible with VAD (Fig 2).

The patient was hospitalized for 11 days and was treated with intravenous heparin sodium and symptomatic treatment (antiemetics, fluid hydration). He was released from the neurology department in stable condition, with complete resolution of his vertigo. During 6 months of follow-up, there was no recurrence of symptoms.

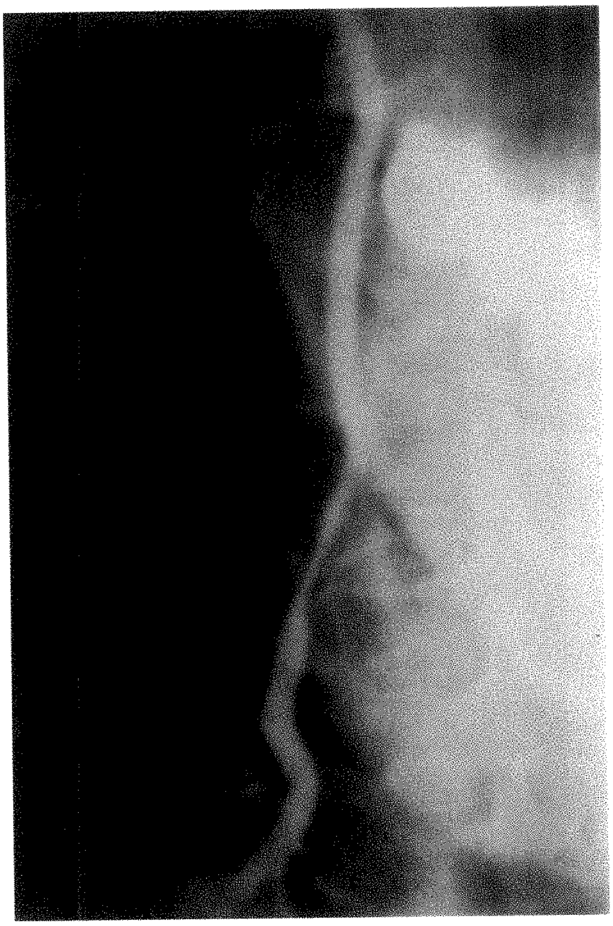

Fig 2. Angiography of neck vessels demonstrates 6.5-cm stenosis of proximal right vertebral artery at C6-7 level, consistent with dissection. Posterior inferior cerebellar artery is normal. Right and left common carotid and left vertebral arteries are normal.

DISCUSSION

The differentiation between central and peripheral origins of vertigo is usually difficult on the basis of clinical examination alone. The differential diagnosis is generally based on documentation of concomitant auditory and neurologic symptoms, enabling discrimination between central and peripheral vertigo. Therefore, a full audiovestibular evaluation is essential, but may not be adequately sensitive for anterior and posterior fossa lesions. The clinician has to distinguish between a benign peripheral vestibular disease and a more serious and dangerous situation.[11]

Vertebral artery dissection is one of the most important causes of posterior circulation stroke in healthy young and middle-aged adults. The distinction that is made between spontaneous dissection and dissection associated with minor trauma appears to be arbitrary in literature reports.[4,12-14] Spontaneous VAD is only infrequently reported, and usually, clear signs of brain stem or cerebellar syndrome are observed. Dissection of the vertebral artery probably occurs more often than is currently recognized.[1,7]

Obstruction of the vertebral artery can influence the blood supply to the brain stem and to the inner ear. The blood supply to the cerebellum occasionally includes the posterolateral medulla territory that contains the vestibular nuclei.[15] These nuclei are located in the fourth ventricle floor in the posterolateral area of the medulla.

In 1967, Bostrom and Liliequist[8] reported on a case of renal artery and vertebral artery dissections in a patient who died with renal failure, and there were no notes of any neurologic signs or symptoms. Hugenholtz et al[16] reported in 1982 on a case of vertebral artery dissecting aneurysm at the level of C4-5, in which the patient presented with headache and neck pain but had no neurologic deficits. Caplan et al[7] reported in 1985 on 5 patients with VAD. All had headache and neck or occipital pain, and 4 had neurologic abnormalities, including elements of lateral medullary syndrome. Chiras et al[17] reported in 1985 on 15 patients with spontaneous VAD, 5 of whom had bilateral dissections. Ten patients had suffered strokes in the vertebrobasilar distribution, consisting of Wallenberg's syndrome in 4 cases. In 8 cases, neck pain preceded a stroke, and in 4 cases there was posterior headache. Most of the patients were female.

In the present report, a young adult presented with acute severe vertigo accompanied by strong nystagmus that was of a horizontal-rotatory type, typical of the nystagmus of a peripheral vestibular lesion. The patient's examination findings were otherwise normal, including normal audiological tests. Because of his condition (severe vertigo), electronystagmography could not be done, and was postponed ultimately, when the MRI and angiography indicated the correct diagnosis.

To our knowledge, this is the only reported case of spontaneous VAD presenting with acute vertigo as the only symptom. Recently, there was a report of a young patient who developed sudden vertigo and was considered to have VAD, which gave rise to lower motor neuron deficit.[18] This patient had an acute vestibular crisis, left neck pain, and headache. Before the onset of symptoms, he had tinnitus and neck pain, which were followed by complete hearing loss in the left ear. His examination revealed a mild unilateral left-hand drift, and abnormal heel-shin test. This is the only case in which the main symptoms of VAD were otologic. In our patient, the only symptom of VAD was the vestibular manifestation.

Occlusion of the vertebral artery may result in cerebellar infarction without accompanying brain stem involvement, and in these cases, typical brain stem signs do not occur. The presence of prominent cerebellar signs (eg, ataxia of gait and extremities) is the key to the diagnosis.[19,20]

In the present case, selective damage to the patient's vestibular labyrinth is unlikely, as one would have expected hearing loss due to the higher sensitivity of the cochlea to a vascular insult. Therefore, the vertigo in the above patient is most probably due to his brain stem lesion, that is, an injury to the vestibular nuclei.

The present case is unique in that it was characterized by a benign course and symptomatology that mimicked the peripheral type of vertigo. It was not accompanied by any other neurologic signs. Migrainous ischemia can mimic VAD, with vasospasm causing irregular stenosis of the vertebral artery.[21,22] Vertigo alone has not been reported as mimicking VAD, and if vertigo was reported, it came with other symptoms.

The evaluation of a patient presenting with acute severe vertigo must include a full neurologic and otolaryngological examination. If there are no other clinical symptoms but the patient looks very ill, and there are no other explanations for the severe vertigo, an enhanced MRI must be considered to rule out a cerebellar infarction. It should be remembered that one of the differential diagnoses of vestibular neuronitis is cerebellar infarction. Due to the potential catastrophic outcome of this condition, a high level of suspicion should be maintained by both neurologists and otolaryngologists in patients with sustained vertigo, even if no neurologic abnormalities are observed.

Because neck torsion can affect vertebral artery blood flow and potentially contribute to the dissection, limitation of neck mobility by means of a soft cervical collar and restricted activity for a few weeks has been recommended.[1]

REFERENCES

1. Hart RG. Vertebral artery dissection. Neurology 1988;38:987-9.

2. Caplan LR, Baquis GD, Pessin MS, et al. Dissection of the intracranial vertebral artery. Neurology 1988;38:868-77.

3. Mokri B, Houser OW, Sandok BA, Piepgras DG. Spontaneous dissections of the vertebral arteries. Neurology 1988;38:880-5.

4. Hart RG, Easton JD. Dissections. Stroke 1985;16:925-7.

5. Robertson JT. Neck manipulation as cause of stroke [Editorial]. Stroke 1981;12:1.

6. Brgousslavsky J, Despland PA, Regli F. Spontaneous carotid dissection with acute stroke. Arch Neurol 1987;44:137-40.

7. Caplan LR, Zarins CK, Hemmati M. Spontaneous dissection of the extracranial vertebral arteries. Stroke 1985;16:1030-8.

8. Bostrom K, Liliequist B. Primary dissecting aneurysm of the extracranial part of the internal carotid and vertebral arteries: a report of three cases. Neurology 1967;17:179-86.

9. Schellhas KP, Latchaw RE, Wendling LR, Gold LHA. Vertebrobasilar injuries following cervical manipulation. JAMA 1980;244:1450-3.

10. Tramo MJ, Hainline B, Petito F, Lee B, Caronna J. Vertebral artery injury and cerebellar stroke while swimming: case report. Stroke 1985;16:1039-42.

11. Disher MJ, Telian SA, Kemink JL. Evaluation of acute vertigo: unusual lesions imitating vestibular neuritis. Am J Otol 1991;12:227-31.

12. Bradac GB, Kaernback A, Bolk-Weischedel D, Finck GA. Spontaneous dissecting aneurysm of cervical cerebral arteries. Report of six cases and review of the literature. Neuroradiology 1981;21:149-54.

13. Goldstein SJ. Dissecting hematoma of the cervical vertebral artery. Case report. J Neurosurg 1982;56:451-4.

14. Katirji MB, Reinmuth OM, Latchaw RE. Stroke due to vertebral artery injury. Arch Neurol 1985;42:242-8.

15. Osborn AG. Diagnostic neuroradiology. St Louis, Mo: Mosby, 1994.

16. Hugenholtz H, Pokrupa R, Montpetit VJA, Nelson R, Richard MT. Spontaneous dissecting aneurysm of the extracranial vertebral artery. Neurosurgery 1982;10:96-9.

17. Chiras J, Maciano S, Vega Molina J, Touboul J, Poirier B, Bories J. Spontaneous dissecting aneurysm of the extracranial vertebral artery (20 cases). Neuroradiology 1985;27:327-33.

18. Strome SE, Hartshorn DO, Carroll WR, Shepard N, Disher MJ. Otologic manifestations of vertebral artery dissection. Otolaryngol Head Neck Surg 1997;116:262-4.

19. Duncan GW, Patier SW, Fisher CM. Acute cerebellar infarction in the PICA territory. Arch Neurol 1975;32:364-8.

20. Sypert GW, Alvord EC. Cerebellar infarction. Arch Neurol 1975;32:357-63.

21. Fisher CM. Unusual vascular events in the territory of the posterior cerebellar artery. Can J Neurol Sci 1986;13:1-7.

22. Solomon GD, Spaccavento LJ. Lateral medullary syndrome after basilar migraine. Headache 1982;22:171-2.

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ORBITAL BLOWOUT FRACTURE WITH PERSISTENT MOBILITY DEFICIT DUE TO FIBROSIS OF THE INFERIOR RECTUS MUSCLE AND PERIMUSCULAR TISSUE

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A case of orbital blowout fracture accompanied by fibrosis of the inferior rectus muscle resulting in an irreversible orbital mobility deficit is reported. An 8-year-old girl with an orbital blowout fracture was treated with steroids for 10 days, as with other cases in our department. She exhibited a disturbance of vertical eye movement and a positive forced duction test result. Although surgery was performed on day 13, and on day 27 due to poor recovery after the first operation, almost no improvement of the ocular movement was noted. The results of a traction test, performed during the second operation, suggested that the inferior rectus muscle had adhered to the periosteum. Magnetic resonance imaging performed 3 days after the second operation revealed fibrosis of the inferior rectus muscle and perimuscular tissue, resulting in an irreversible disturbance of the vertical ocular movement. The present findings suggest that the need for and timing of surgery in patients with blowout fractures should be determined on an individual basis.

KEY WORDS — blowout fracture, fibrosis, inferior rectus muscle, magnetic resonance imaging.

INTRODUCTION

The indications for and timing of surgery for orbital blowout fractures are controversial.[1-6] At our institution, we have typically performed surgery only if eye movement does not improve after about 2 weeks of conservative therapy.[7] Surgery was performed in the present case 13 days after the initial injury, but no improvement in the patient's mobility deficit was observed; hence, earlier surgical intervention may be indicated in some patients with orbital blowout fractures.

CASE REPORT

An 8-year-old girl lost consciousness after falling from a horizontal bar at her school on September 21, 1994, and was referred to a nearby hospital. A computed tomography scan revealed subdural effusion, and she was hospitalized for 2 days for neurologic observation. She reported double vision, and disturbed movement of the left eye was detected during hospitalization. After being discharged, she went to the nearest eye clinic 3 days after the injury and was then referred to our department. A blowout fracture on the left side was diagnosed based on disturbances of the upward and downward gaze and a positive result on a forced duction test. Although surgical therapy was recommended, she was treated conservatively with steroids because of the persistence of subdural effusion. Since conservative therapy caused no improvement in eye movement, she underwent surgery by the transantral approach 13 days after the injury. Orbital tissue that had prolapsed through the linear fracture along the infraorbital groove was replaced, but a postoperative traction test revealed only slight relief of the restricted eye movement, and spontaneous movement was not improved, except for a slight improvement in the downward gaze. Therefore, the orbital floor was surgically exposed by the transeyelid approach 14 days after the first operation. When the periosteum of the orbit was lifted, the orbital tissue was found to have been replaced properly. No orbital soft tissue had prolapsed into the maxillary sinus, but scar formation was noted at the site of the orbital periosteum through which the tissue appeared to have prolapsed initially. A traction test revealed severe restriction, even when the orbital periosteum was raised; and the periosteum showed marked tension at the site of the scarring when the inferior rectus muscle was pulled, as if the muscle had adhered tightly to the periosteum. Because further manipulation inside the orbital periosteum was not considered feasible, the operation was terminated. There was almost no improvement in eye movement postoperatively. A magnetic resonance imaging (MRI) scan obtained 3 days after the surgery revealed an intensity change and a thickening of part of the inferior rectus muscle, suggesting degeneration or fibrosis, at the site of muscle adherence to the periosteum (Fig 1). An MRI scan obtained 5 months

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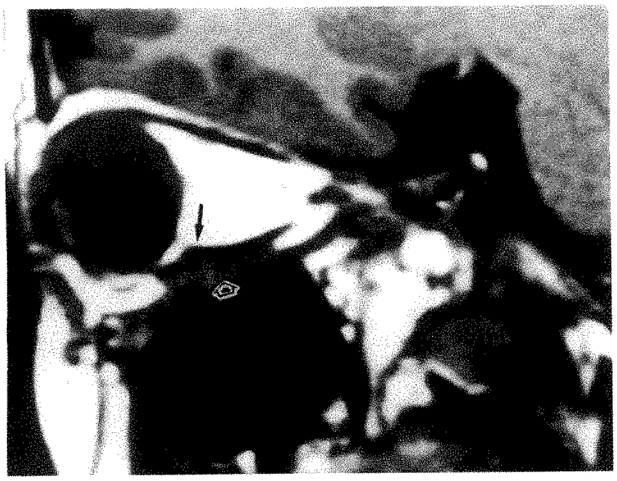

Fig 1. Sagittal T1-weighted magnetic resonance image obtained 30 days after injury (17 days after first operation and 3 days after second) shows kink in anterior portion of inferior rectus muscle (thin black arrow) and isointensity signal suggesting fibrosis between inferior rectus muscle and periosteum (open, wide arrow). No prolapse of orbital contents is recognized.

after the second operation showed similar findings, with a kink in the muscle (Fig 2).

DISCUSSION

Consensus is lacking on the optimal treatment for blowout fractures. Some authors recommend early surgical therapy,[1-3] while others advocate surgery only when conservative therapy has been ineffective.[4,5,8] A third opinion is that optimal recovery can be obtained with conservative therapy alone.[6] We used to perform surgery when no improvement was observed after about 10 days of conservative therapy. Surgery was performed in the present case after 10 days of conservative therapy (13 days after the injury). However, MRI performed 30 days after the injury showed findings suggestive of fibrosis of the inferior rectus muscle and its adhesion to the periosteum. At the initial operation, the orbital contents, including the inferior rectus muscle, were found to have prolapsed into the maxillary sinus through the narrow fracture, causing severe entrapment. The MRI findings suggested that the inferior rectus muscle had gradually degenerated before surgery was performed. Adhesion of the muscle to the periosteum, in addition to fibrosis of the muscle and surrounding tissue, appeared to be responsible for the patient's irreversible disturbed eye movement.

Diplopia persists in a significant number of patients following surgical treatment of a blowout fracture of the orbital floor, even when surgery is performed within 15 days after the traumatic incident.[9] Diplopia is probably due to a myogenic or neurogenic cause and may require surgical correction, depending upon the degree of vertical dissociation.[9] Lisman et al[10] have reported that diplopia becomes permanent if a Volkmann's-type ischemic contracture of the inferior rectus muscle is left untreated. They also found that elderly patients, hypotensive patients, patients with peripheral vascular disease, and patients with small fractures were more likely to develop high compartment pressures, with resultant muscle fibrosis, and suggested that surgical intervention is a more prudent approach than medical management in high-risk patients with small blowout

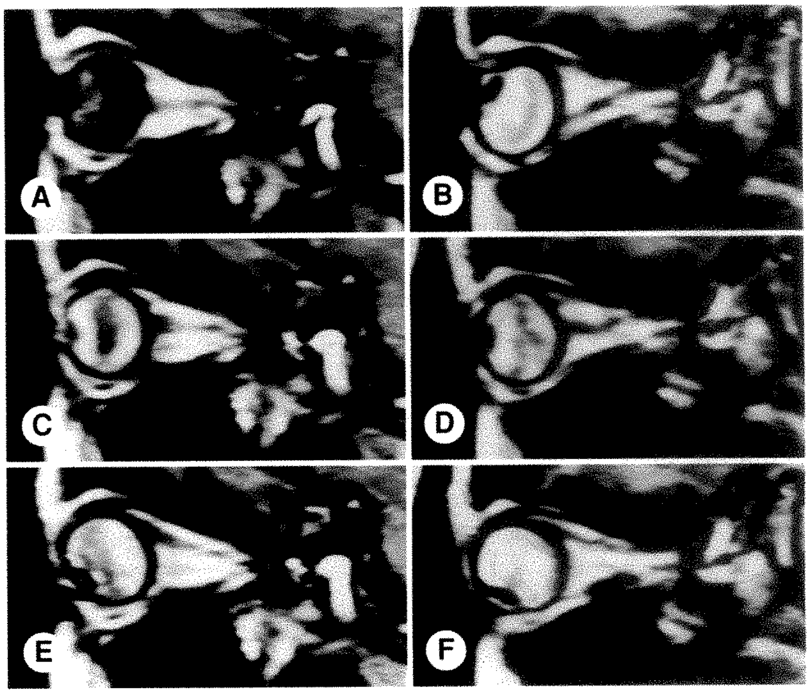

Fig 2. Sagittal T1-weighted magnetic resonance images obtained 6 months after injury. A,C,E) Affected side. B,D,F) Healthy side. A,B) Upward gaze. C,D) Primary position. E,F) Downward gaze. Globe on affected side still shows disturbance of vertical gaze. Kink of inferior rectus muscle on affected side is still shown, with no obvious change in shape of muscle on eye movement.

fractures. Irreversible muscle damage has been induced experimentally after only 5 to 6 hours of ischemia.[10] Our patient had a positive result on the traction test, although the fractured bone showed only minimum deviation. Orbital contents were incarcerated tightly in the floor of the orbital fracture. Therefore, surgery should have been performed soon after the injury. Because of the flexibility of the bone matrix and the thick periostea, fractures tend to be linear in children,[11] and the orbital tissue can become entrapped in the narrow linear space, causing severe constriction. The findings in the present case suggest that the indications for and timing of surgical intervention for blowout fractures should be determined on a case-by-case basis.

CONCLUSION

Our patient had a poor outcome because surgery was delayed for 13 days after an orbital blowout fracture. Prolonged constriction of the orbital tissue probably caused fibrosis of the inferior rectus muscle and the surrounding tissue, as well as adhesion of the muscle to the periosteum, resulting in an irreversible disturbance of the vertical ocular movement. Surgery should have been performed as early as possible in this case. The indications for and timing of surgery for blowout fractures should be determined on an individual basis.

REFERENCES

1. Chen JM, Zingg M, Laedrach K, Raveh J. Early surgical intervention for orbital floor fractures: a clinical evaluation of lyophilized dura and cartilage reconstruction. J Oral Maxillofac Surg 1992;50:935-41.
2. Reeh MJ, Tsujimura JK. Early detection and treatment of blowout fracture of the orbit. Am J Ophthalmol 1966;62:79-82.
3. Friesenecker J, Dammer R, Moritz M, Niederdellmann H. Long-term results after primary restoration of the orbital floor. J Craniomaxillofac Surg 1995;23:31-3.
4. Millman AL, Della RR, Spector S, Leibeskind AL, Messina A. Steroids and orbital blowout fractures — a new systematic concept in medical management and surgical decision-making. Adv Ophthalmic Plast Reconstr Surg 1987;6:291-300.
5. Emery JM, Von Noorden GK, Schlernitzauer DA. Orbital floor fractures: long-term follow-up of cases with and without surgical repair. Trans Am Acad Ophthalmol Otolaryngol 1971;75:802-12.
6. Putterman AM, Stevens T, Urist MJ. Nonsurgical management of blow-out fractures of the orbital floor. Am J Ophthalmol 1974;77:232-9.
7. Yoneda T, Sekitani T, Hiyoshi M, Okinaka Y, Imate Y, Ueki A. Blowout fracture of the orbit. Report of twenty-five cases [in Japanese]. J Otolaryngol Jpn 1985;88:1162-7.
8. Miura M, Sekitani T. Blowout fractures of the orbit. Bull Yamaguchi Med Sch 1992;39:79-82.
9. Harley RD. Surgical management of persistent diplopia in blowout fractures of the orbit. Ann Ophthalmol 1975;7:1621-6.
10. Lisman RD, Smith BC, Rodgers R. Volkmann's ischemic contractures and blowout fractures. Adv Ophthalmic Plast Reconstr Surg 1987;7:117-31.
11. Takizawa Y, Yabe H, Kimura C. Evaluation of 21 cases of blowout fracture in children [in Japanese]. Folia Ophthalmol Jpn 1993;44:355-8.

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IMAGING CASE STUDY OF THE MONTH

PEDIATRIC ETHMOID CHONDROMA INCIDENTALLY FOUND DURING EVALUATION OF A THIRD CRANIAL NERVE PALSY

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INTRODUCTION

Chondromas occur infrequently in the head and neck and are rare in children. Following is a case report of an ethmoid chondroma in an 18-month-old girl, along with a review of the literature. This case has 2 features that are unusual for sinonasal chondromas. First, the patient is young for this disease. Kilby and Ambegaokar's[1] review of the world literature for 135 years identified only 7 cases of sinonasal chondroma in children less than 10 years of age. Second, the ethmoid tumor was found by chance through workup of a presumed congenital oculomotor palsy.

CASE REPORT

An 18-month-old girl presented to the Columbia Presbyterian Medical Center (CPMC) with the diagnosis of a right third cranial nerve palsy made soon after birth. She was followed up at an outside hospital by an ophthalmologist who treated her with daily patching. At 18 months, a magnetic resonance imaging (MRI) study was obtained to evaluate the eye, and this revealed a mass in the right ethmoid sinus. She was then referred to CPMC for further evaluation and treatment.

The patient's history was significant for occasional rhinitis and multiple episodes of otitis media. She had generally normal development, and the history was otherwise unremarkable. Physical examination showed a mildly proptotic and inferiorly displaced right eye; the pupils were slightly unequal, with the right pupil larger than the left. Right ocular examination revealed severe impairment of up-gaze and adduction, moderate impairment of down-gaze, normal abduction, and the presence of upper lid ptosis. Fiberoptic examination of the nose showed a fleshy pink mass that was of soft tissue consistency and located anterior to the middle turbinate. Otoscopic examination was significant for a retracted right eardrum with purulent fluid behind the tympanic membrane.

A computed tomography (CT) scan demonstrated a 1.6 × 1.2-cm soft tissue mass in the right ethmoid sinus and nasal antrum that was noted to displace the right medial rectus muscle laterally (Fig 1). Additionally, bone erosion was noted on the floor of the anterior cranial fossa at the frontal recess in the region of the cribriform plate. Another MRI study confirmed that the ethmoid mass did not appear to be contiguous with the brain (Fig 2).

An endoscopic biopsy of the mass was performed. Histologic examination revealed a multilobulated lesion composed of cytologically benign-appearing spindle cells. The tissue was composed of round to

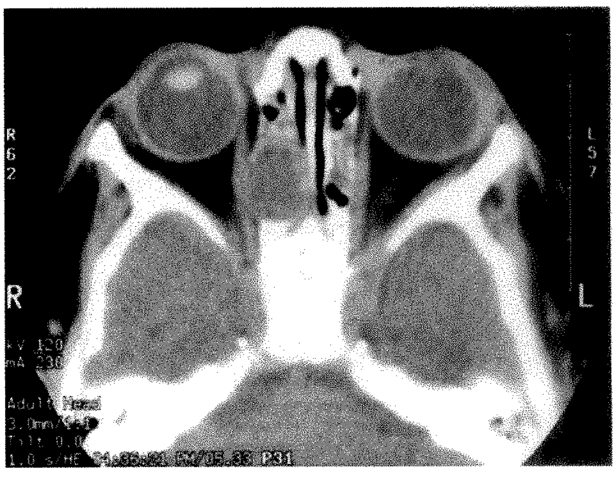

Fig 1. Computed tomography scan in axial plane demonstrating 1.6 × 1.2-cm soft tissue mass in right ethmoid sinus. Note thinning of medial wall of right orbit and lateral deviation of medial rectus muscle caused by mass.

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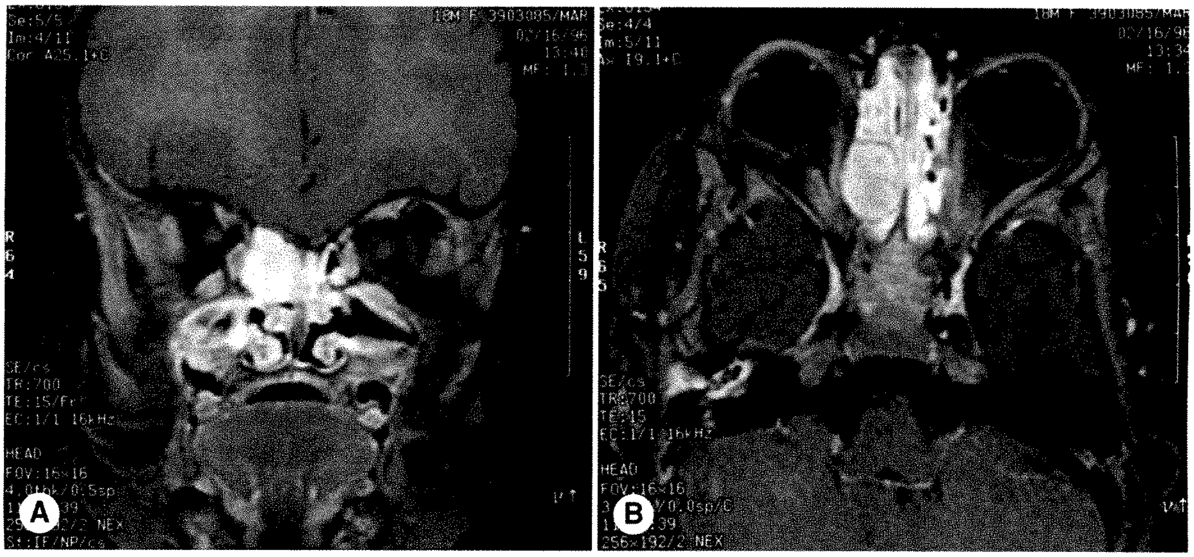

Fig 2. T1-weighted magnetic resonance imaging with gadolinium enhancement. A) Coronal plane. Mass in right ethmoid sinus is in close proximity to brain, but does not appear to be contiguous with it. B) Axial plane. Mass is visualized in posterior right ethmoid sinus with mild heterogeneous enhancement. No inflammatory changes are seen in surrounding tissue. There is expansion of right lamina papyracea laterally into orbit, with thinning of right ethmoid sinus. This mass abuts right medial rectus and produces lateral deviation of muscle, but shows no invasion or extension into muscle.

ovoid nuclei in lacunar spaces, suggesting cartilaginous differentiation with little pleomorphism and no mitotic figures (Fig 3). In some areas, lesional cells were seen to lie within a myxoid stroma. Immunohistochemical studies were positive for S-100 protein, consistent with a cartilaginous tumor, and for vimentin, consistent with a mesenchymal lesion. Negative cytokeratin and epithelial membrane antigen stains were features seen against an epithelial neoplasm; a negative desmin stain made the diagnosis of a muscle tumor unlikely. The lesion was diagnosed as a myxoid chondroma, and surgical removal of the mass was planned.

On the basis of the localized nature of the mass, the consistency of the tumor, its pathology, and cosmetic concerns, an intranasal endoscopic approach to perform a right total ethmoidectomy and maxillary antrostomy was combined with an extradural bifrontal craniotomy. During the initial surgical approach, the right frontal lobe was retracted away from the orbital roof in an extradural fashion. The lesion was noted to have eroded through the posterior-medial aspect of the orbital roof at the level of the cribriform plate. The tumor was intimately involved with the right olfactory nerve, but did not appear to go intradural. The tumor was carefully dissected free of the dura, which showed no evidence of invasion. The right olfactory nerve was sacrificed near its entry to the dura, and the dura was primarily repaired

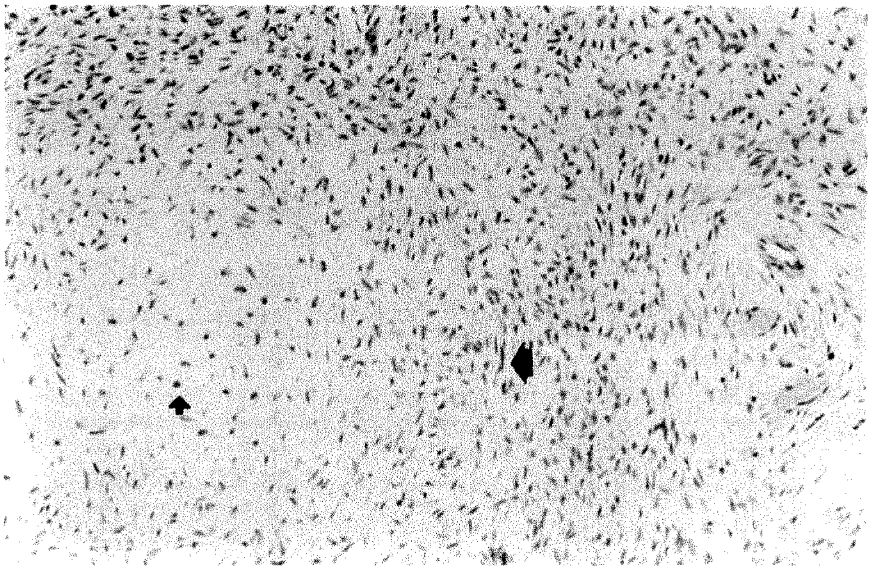

Fig 3. Hematoxylin and eosin–stained section of mass (original ×200). Cytologically benign-appearing spindle-shaped cells (large arrow) are noted to be adjacent to area of definite cartilaginous differentiation, with round to ovoid nuclei found in clear-cut lacunar spaces (small arrow).

with several 5-0 silk sutures. There was no evidence of cerebrospinal fluid (CSF) leakage after this was performed. At the completion of the surgery, a vascularized pericranial scalp flap was brought down to cover the cranial defect and isolate the brain from the ethmoid sinus.

Postoperatively, the patient was maintained on a spinal drain to allow CSF diversion during the acute healing phase. The patient did well until 1 week after surgery, when she developed a right-side rhinorrhea. This was treated with endoscopic repair utilizing a piece of temporalis fascia and muscle and CSF diversion utilizing a spinal drain. The patient subsequently did well, with resolution of the leak, and she recovered without further incident.

She has been followed up for 2$^1/_2$ years since the initial surgery, and follow-up examinations and MRI show no evidence of tumor recurrence. The patient recently underwent surgery to explore the right orbit and attempt cosmetic and functional improvement of the right eye. Exploration revealed fibrosis of all of the extraocular muscles and orbital tissues; this congenital problem can mimic third nerve palsy.

DISCUSSION

A chondroma is a benign neoplasm of cartilage.[2] These tumors are rare in the head and neck, with an estimated 10% occurrence in this region. When present in the head and neck, they are typically found within the larynx or paranasal sinuses.[3] Of nonepithelial tumors within the nasal cavity and paranasal sinuses, Fu and Perzin[4] estimated that only 7% are chondromas.

The distribution of these rare tumors was defined in an extensive review of the world literature for a 135-year period by Kilby and Ambegaokar.[1] They found that sinonasal chondromas have an equal sex distribution and are unusual in children. While the age of presentation ranged from 8 months to greater than 70 years old, only 5% of cases were found to occur in patients less than 10 years of age. The sites of origin were, in descending order, the ethmoids and nasal cavity (50%), the maxilla (18%), the nasal septum (17%), the hard palate (6%), the sphenoid sinus and eustachian tube (6%), and the alar cartilage (3%).[1]

Clinically, chondromas are typically painless, asymptomatic lesions that are found incidentally during a routine nasal examination. When symptoms are present, they usually include nasal obstruction and stuffiness. Chondromas rarely cause local signs such as soft tissue swelling and proptosis.[1,2] The review of Kilby and Ambegaokar[1] found that only 5 of 128 patients with sinonasal chondromas developed blindness.

Grossly, lesions are typically nodules that measure less than 3 cm and that project into the lumen of the nasal cavity or nasopharynx. Histologically, they are composed of well-differentiated lobulated hyaline cartilage and often demonstrate a minor degree of cellular atypia.[4,5] Recognizing a chondroma as a benign lesion can be challenging. According to Batsakis et al,[6] the histopathologic distinction between a chondroma and a low-grade chondrosarcoma is notoriously difficult. Many of the fine structural features of low-grade chondrosarcoma cells are also found in cells of normal hyaline cartilage.[6]

The treatment of choice is complete surgical removal with an adequate margin of normal tissue to prevent recurrence and possible malignant degeneration.[5] While the vast majority of tumors do not respond to radiotherapy,[3] local excision is curative in almost all cases.[7] Due to the location of this lesion, and the strong consideration that the mass had either a dural or intradural component, it was felt that the tumor would be best addressed by utilizing a combined approach. This combined approach affords an excellent opportunity to visualize any extent of tumor and minimize risk of dural or cortical injury, which would be difficult to repair from a purely intranasal endoscopic approach.

The patient described in this report had an unusual presentation of a rare disease. Five percent of sinonasal chondromas occur in patients less than 10 years old. They are usually asymptomatic or cause nasal stuffiness. Other entities to consider in the differential diagnosis of the ethmoid tumor include both benign and malignant neoplasms. Benign masses frequently found in the paranasal sinuses include mucoceles, polyps, fibrous dysplasia, ossifying fibromas, giant cell tumors, and hemangiomas. Malignant lesions such as rhabdomyosarcomas, carcinomas, lymphomas, and leukemias need to be considered in any case of a pediatric paranasal sinus mass.

The present case of ethmoid chondroma was noted by chance through evaluation of a presumed congenital oculomotor palsy. Recent surgical examination of the orbit revealed fibrosis of the extraocular muscles rather than third cranial nerve palsy as the cause of the patient's eye findings, and points out the difficulty of determining the cause of extraocular muscle problems in infants. While the ethmoid chondroma was not related to the eye problem, the case highlights the benefits of imaging for difficult cases, and the benefits of a combined surgical approach for removal of benign ethmoid masses.

REFERENCES

1. Kilby D, Ambegaokar AG. The nasal chondroma. 2 case reports and a survey of the literature. J Laryngol Otol 1977;91: 415-26.

2. Unni KK. Bone tumors. In: Sternberg SS, Antonioli DA, Carter D, Mills SE, Oberman HA, eds. Diagnostic surgical pathology. New York, NY: Raven Press, 1994:271-322.

3. Jones HM. Cartilaginous tumours of the head and neck. J Laryngol Otol 1973;87:135-51.

4. Fu YS, Perzin KH. Non-epithelial tumors of the nasal cavity, paranasal sinuses, and nasopharynx: a clinicopathologic study: III. Cartilaginous tumors (chondroma, chondrosarcoma). Cancer 1974;34:453-63.

5. Hyams VJ. Pathology of the nose and paranasal sinuses. In: English GR, ed. Otolaryngology. Vol 2. Philadelphia, Pa: Lippincott-Raven, 1983:1-128.

6. Batsakis JG, Solomon AR, Rice DH. The pathology of head and neck tumors: neoplasms of cartilage, bone, and the notochord, part 7. Head Neck Surg 1980;3:43-57.

7. Konrad HR. Nasal neoplasms. In: English GR, ed. Otolaryngology. Vol 5. Philadelphia, Pa: Lippincott-Raven, 1989:1-11.

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IMAGING CASE STUDY OF THE MONTH

ISOLATED UNILATERAL FIBROUS DYSPLASIA OF THE SPHENOID SINUS

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INTRODUCTION

Fibrous dysplasia as a clinical term was introduced by Lichtenstein[1] as early as 1938. The disease is characterized by a slowly progressive replacement of cancellous bone by abnormal fibrous tissue that histologically represents various stages of bone metaplasia.[2] The proportions of the fibrous and osseous tissues vary, occasionally even in the same bone.[3,4] The development of the disease lasts for years and presents with deformity or mild symptoms. This locally destructive disease can occur in association with endocrine abnormalities such as Albright's syndrome (precocious puberty, cutaneous pigmented patches over the involved bones) or as an isolated clinical entity. Rarely, fibrous dysplasia is associated with other bone pathological changes such as aneurysmal bone cyst inside the paranasal sinuses[5] or mucocele.[6] The cause of the disease, however, remains unknown.

Ramsey et al[7] offered a classification of fibrous dysplasia into 3 types: type 1, characterized by single or multiple lesions in 1 and the same bone (monostotic type); type 2, characterized by multiple lesions involving more than 1 bone (polyostotic); and type 3, characterized by dissemination of pathological bone changes connected to other disturbances such as Albright's syndrome. There are also classifications according to histologic and radiologic findings.[8,9] In terms of the predisposing sites of its clinical appearance, craniofacial fibrous dysplasia is most frequently located in the mandible and maxilla.[2,10-12] The paranasal sinuses are rarely involved. The most common locations are the maxillary and, less frequently, ethmoidal sinuses.[13] To the best of our knowledge, an isolated, unilateral fibrous dysplasia of the sphenoid sinus has not been reported so far in the literature.

CASE REPORT

A 21-year-old man with a history of recent onset of severe headaches was evaluated by computed tomography (CT) and magnetic resonance imaging (MRI). An undefined "mass" was found within the remarkably enlarged right sphenoid sinus (Figs 1 and 2). To exclude a possible relationship between the mass and the internal carotid artery, as well as the right cavernous sinus, arteriography of both external and internal carotid arteries was performed, and the results were negative. An endoscopic surgical approach to the right sphenoid sinus was performed.

The posterior third of the middle turbinate had to be removed to make the anterior sphenoid wall more visible. However, despite the relatively good visi-

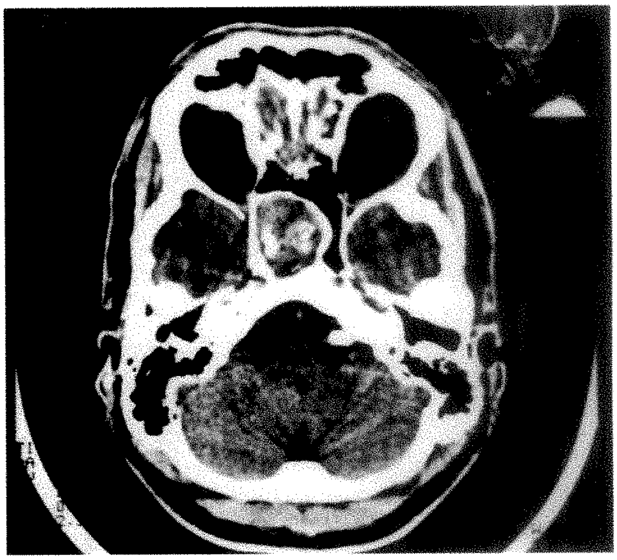

Fig 1. Enhanced axial computed tomogram of sphenoid sinus showing sclerosis and emphasized expansion of right sphenoid sinus. Surrounding bony structures seem to be of normal appearance. Computed tomography appearance of sinus content is characterized by heterogeneous density (ground-glass appearance). Isolated, bone-like particles of irregular, bizarre shape inside involved sinus not only closely resembled osteosarcoma's spicules, but also suggested aspergilloma.

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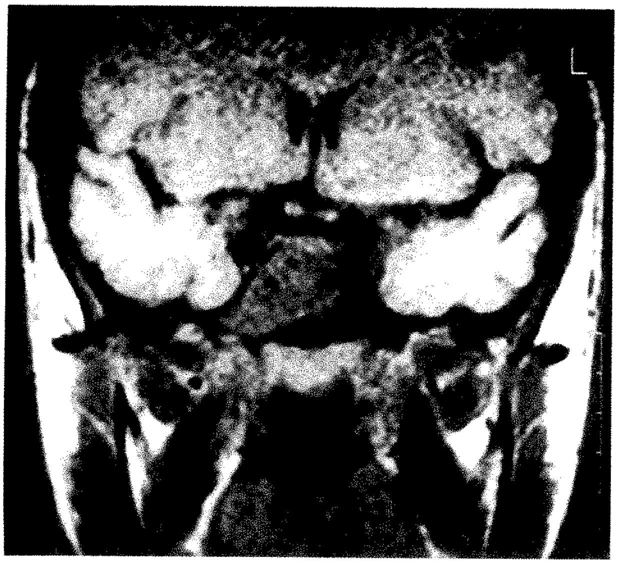

Fig 2. Magnetic resonance coronal image showing bizarre formation of heterogeneous density in projection of right sphenoid sinus. No signal characteristics suggesting calcification can be seen within mass.

bility and all surgical endeavours, the crucial maneuver of opening the anterior wall by a direct approach through the sphenoethmoid recess remained unsuccessful. Hence, an additional, more classic endoscopic approach to the sphenoid sinus through the ostiomeatal complex and posterior ethmoid was performed. This helped ensure that the site of previous attempts in opening the sphenoid sinus was correct. Finally, it became obvious that we were dealing with "bone in bone": after the partial removal of an unexpected bony "shell," an unusual bony-fibrous tissue was found behind it. The tissue was very adherent to the adjacent sinus walls, making removal, particularly in the zones of the optic nerve and carotid artery, very tricky. Nevertheless, a vast majority of the suspect tissue was removed. Histopathologic analysis of the frozen sections revealed fibrous dysplasia. Proliferative, well-vascularized fibrous stroma surrounding the irregularly shaped trabeculae of woven bone without osteoblastic activity was found. Its appearance was intraluminal, suggesting the origin of this unusual mass to be from the sphenoid sinus septum (Fig 3). Fifteen months after the surgery, the patient is doing well.

DISCUSSION

Fibrous dysplasia develops mostly during the first 2 decades of life and usually regresses at puberty, although it may continue to grow slowly in adulthood.[14,15] It seems that this was the case in our patient.

Fibrous dysplasia in an "active phase," ie, prior to its presumed regression in adolescence, can show a very dense vascularity. The increased vascularity of active lesions may cause symptomatic arteriovenous shunting.[16] Fortunately, we did not find any arteriovenous shunting during arteriography of the internal and external carotid arteries. Further, some authors state that even in symptomatic patients with biopsy-proved fibrous dysplasia, because of a dense vascularity the surgery should be delayed when feasible, until the growth of fibrous dysplasia slows or ceases.[17]

With craniofacial involvement, visual loss may be caused by compression of the optic nerve[18] or chiasm[19] by dysplastic bone, or by hemorrhage into dysplastic bone.[20] In our patient we did not find any clinical, CT, or MRI signs of involvement of other structures, except the right sphenoid sinus. To the best of our knowledge, this is the first report of an isolated, unilateral fibrous dysplasia of the sphenoid

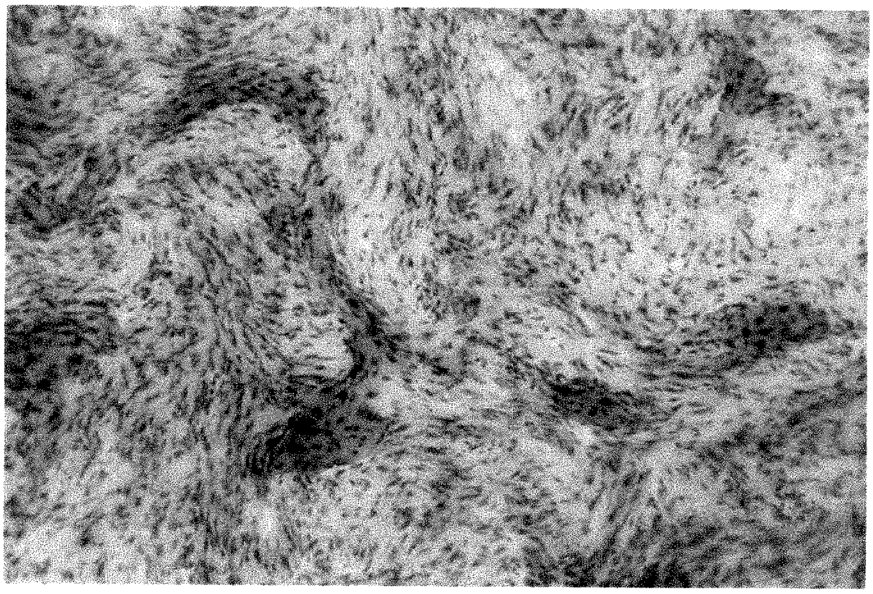

Fig 3. Proliferative, well-vascularized fibrous stroma surrounding irregularly shaped trabeculae of woven bone without osteoblastic activity (H & E, original ×100).

sinus. The most similar case was that described by Mueller et al[10] in 1992, but the disease was not entirely isolated, since they found the ipsilateral great wing of the sphenoid bone to be involved as well.

Concerning the differential diagnosis prior to surgery, it must be emphasized that radiologic signs, although they can be very characteristic, are not always easy to recognize. Fries[8] has classified craniofacial fibrous dysplasia into 3 radiographic patterns: pagetoid, sclerotic, and cystlike. The pagetoid form resembles Paget's disease, with remarkable bone expansion and alternating zones of radiodensity and radiolucency. This type more frequently appears in patients over 30 years of age, and the symptoms can last for more than 15 years on average. The sclerotic type demonstrates a homogeneous, severe density, again accompanied by bone expansion, whereas the cystlike pattern is usually a single, well-defined radiolucent lesion, round or oval in shape, edged by a thin, sclerotic margin clinically resembling an eggshell. Both are frequently seen in children or younger adults (less than 25 years of age) who have had symptoms for less than 3 years.

Fries'[8] classification, however, was established on the basis of conventional radiography. Computed tomography diagnosis has introduced the term "ground glass–like lesion." It usually measures 70 to 130 Hounsfield units (HU), contrary to other lesions, such as osteomyelitis and neoplasms, which usually have lower attenuations of 20 to 40 HU.[9] The bonelike spicules, characteristic of osteosarcomatous lesions, must be differentiated from aspergilloma, since aspergilloma may also present with sporadic opacities of almost bony density.[21] In contrast to osteosarcomatous lesions, these opacities are mostly located in the center of the lesion.

On MRI, fibrous dysplasia displays nonspecific findings, with low signal intensity on T1-weighted images and low, intermediate, or high signal intensity on T2-weighted images.[22-24] It seems that fibrous dysplasia may not be differentiated on the basis of signal intensity alone, ie, by means of standard MRI (Fig 2). This technique may not demonstrate a thin calcific shell and calcifications as effectively as CT (Fig 1).[16] Our patient showed many "characteristic" radiologic and MRI signs, which confused us.

From the histopathologic point of view, woven bone usually predominates in typical fibrous dysplasia, but various amounts of lamellar bone may also be seen.[16] The microscopic differential diagnosis of fibro-osseous clinical entities of the facial bones includes fibrous dysplasia, ossifying fibroma, and cementifying fibroma.[14] They all resemble each other, so it is difficult to distinguish among them. Still, ossifying fibromas are often well delimited and are composed of lamellar bone, lined by osteoblasts. Cementifying fibromas are made up of spherules with interspersed fibroblastic changes; the spherules are interpreted as cementum.

The histology in our case showed an ill-defined lesion made up of proliferating fibroblasts in a compact stroma of interlacing collagen fibers. Irregular C-shaped or Chinese character–shaped trabeculae of the woven bone were scattered throughout the lesion. Osteoblastic rimming was not observed (Fig 3).

In regard to secondary malignant degeneration of fibrous dysplasia, pain and soft tissue extension should be strongly considered, since sarcomatous alterations occur in 0.5% of all patients.[25,26] More than half of these sarcomas are located in the craniofacial region. Osteosarcoma is the most common secondary tumor in fibrous dysplasia.[23]

Finally, there is the problem of how to adequately follow up patients operated on for fibrous dysplasia, since it is difficult to distinguish among recurrent lesions, postoperative and postirradiation inflammation, and secondary sarcomatous degeneration. It seems that a new MRI technique, gadolinium-enhanced dynamic subtraction MRI, may be useful because it can separate the rapid, early vascular phase of enhancement of malignant tumors from the slower, late vascular phase of enhancement of benign lesions and inflammations.[27]

In conclusion, fibrous dysplasia of the sphenoid sinus presents a diagnostic challenge to both clinicians and radiologists. It must be considered in cases of a calcified, thick margin of the enlarged sphenoid sinus and a ground-glass appearance of the mass inside it. Because of the potential for malignant change, vigilant clinical and imaging assessment of fibrous dysplasia with histopathologic correlation is essential.

REFERENCES

1. Lichtenstein L. Polyostotic fibrous dysplasia. Arch Surg 1938;36:874-7.

2. Antonelli JR. Ossifying fibroma of the maxillary sinus. A case report. Ann Dent 1989;48:33-6.

3. Kiehn CL, Desprez JD, Harris AH. Fibrous dysplasia of the facial bones. Am J Surg 1961;102:835-7.

4. Smith JF. Fibrous dysplasia of the jaws. Arch Otolaryngol 1965:81:592-603.

5. Som PM, Schatz CJ, Flaum EG, Lauman TH. Aneurysmal bone cysts of the paranasal sinuses associated with fibrous dysplasia: CT and MR findings. J Comput Assist Tomogr 1991:15:513-5.

6. Garzozi H, Garty J, Kavch Z. Craniofacial fibrous dysplasia complicated by mucocele. The role of radionuclide scinti-

graphic methods in the diagnosis. Ann Ophthalmol 1989;21: 108-10.

7. Ramsey HE, Strong EW, Frazel EL. Fibrous dysplasia of the craniofacial bones. Am J Surg 1968;116:542-7.

8. Fries YW. The roentgen features of fibrous dysplasia of the skull and facial bones: a critical analysis of 39 pathologically proved cases. AJR Am J Roentgenol 1957;77:71-8.

9. Daffner RH, Kirks DR, Gehweiler JA Jr, Heaston DK. Computed tomography of fibrous dysplasia. AJR 1982;139:943-7.

10. Mueller DP, Dolan KD, Yuh WTC. Fibrous dysplasia of the sphenoid sinus. Ann Otol Rhinol Laryngol 1992;101:100-1.

11. Rifai M, Kenawi B. Visual dysfunction: abnormal sign of benign sphenoethmoidal lesions. J Laryngol Otol 1990;104: 217-21.

12. Barat M. Fibrous dysplasia masquerading as chronic maxillary sinusitis. Ear Nose Throat J 1989;42:44-8.

13. Dornhoffer J, Schwager K. Fibrous dysplasia and ossifying fibroma. Two unusual fibro-osseous lesions of the paranasal sinuses. HNO 1995;43:193-6.

14. Fechner RE. Problematic lesions of the craniofacial bones. Am J Surg Pathol 1989;13(suppl 1):117-32.

15. Henry A. Monostotic fibrous dysplasia. J Bone Joint Surg Br 1969;5B:300-6.

16. Shapeero LG, Vanel D, Ackerman L, et al. Aggressive fibrous dysplasia of the maxillary sinus. Skeletal Radiol 1993; 22:563-8.

17. Harrison DFN. Osseous and fibro-osseous conditions affecting the craniofacial bones. Ann Otol Rhinol Laryngol 1984; 93:199-203.

18. Ogusthorpe JD, Gudeman SK. Orbital complications of fibrous dysplasia of the skull. Otolaryngol Head Neck Surg 1987;97:403-5.

19. De Fanti C, Rivier A. Syndrome chiasmatique sur dysplasie fibreuse du sphenoide. Rev Otoneuroophthalmol 1972;44: 339-46.

20. Kurokawa Y, Sohma T, Tsuchita H, Kitami K, Suzuki S, Sohma K. Hemorrhage into fibrous dysplasia following minor head injury — effective decompression for the ophthalmic artery and optic nerve. Surg Neurol 1989;32:421-6.

21. Stammberger H, Jakse R, Beaufort F. Aspergillosis of the paranasal sinuses. X-ray diagnosis, histopathology, and clinical aspects. Ann Otol Rhinol Laryngol 1984;93:251-6.

22. Norris MA, Kaplan PA, Pathria M, Greenway G. Fibrous dysplasia: magnetic resonance imaging appearance at 1.5 Tesla. Clin Imaging 1990;14:211-4.

23. Som P, Bergeron RT. Head and neck imaging. St Louis, Mo: Mosby–Year Book, 1991:109.

24. Utz JA, Kransdorf MJ, Jelinek JSS, Mosser RP Jr, Berrey BH. MR appearance of fibrous dysplasia. J Comput Assist Tomogr 1989;13:845-9.

25. Schwartz DT, Alpert M. Malignant transformation in fibrous dysplasia. Am J Med Sci 1964;247:1-20.

26. Bagatin M, Manojlovic S, Zajc I, Cacinovic-Matic V. Monostotic fibrous dysplasia of the facial bones. Acta Stomatol Croat 1993;27:51-7.

27. Shapeero LG, Vanel D, De Baere T, Valenty M. Gadolinium-enhanced subtraction MRI: new technique for evaluating response of osteosarcoma to chemotherapy. Radiology 1990; 181:227-33.

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CLINICOPATHOLOGICAL CONSULTATION

MICROMETASTASES: HAVE THEY AN IMPACT ON PROGNOSIS?

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Current routine histopathologic methods may fail in detecting lymph node micrometastases, while the introduction of newer, sensitive techniques, such as immunohistochemistry and molecular analysis, can improve their detection. The literature reveals that the presence of micrometastases has clinical and prognostic implications.

A reliable assessment of the number of metastatic lymph nodes depends on the number of nodes removed and examined, the pathological techniques used, and the surgeon's and/or pathologist's skill in dissection and evaluation. Moreover, the sensitivity of current routine histopathologic methods in assessing regional lymph node involvement is imperfect, in that it, like clinical staging, cannot detect the presence within those nodes of single metastatic tumor cells. At present, the non-overt metastatic status of the patient is often assessed incorrectly, because it is routinely studied by histological examination of only 1 or 2 hematoxylin and eosin sections prepared from a representative cut surface of a lymph node. Since most parts of a lymph node are left unexamined, it is very likely that micrometastases are overlooked.[1,2]

A more careful application of traditional pathological techniques (such as histopathologic examination with serial sections and histochemistry) and the introduction of newer, sensitive techniques (such as immunohistochemistry and molecular analysis) can improve detection of "inapparent" (microscopic, occult, obscure, clandestine, molecular, immunohistochemical, undetected) metastases (Table 1), but it is very important to know if such added information will actually have an impact on prognosis. The Figure shows an algorithm regarding these various pathological methods, which are often complementary in detecting micrometastases. Therefore, a lymph node free of metastasis by conventional histologic examination (pN0, hematoxylin and eosin) may show individual malignant cells or a group of them by immunohistochemistry (pN1, immunohistochemistry), and a lymph node N0 by both mentioned techniques may contain isolated tumor cells or small clusters of cells by molecular analysis (pN1, molecular analysis). Immunohistochemistry and molecular analysis also are capable of converting the status of nodes from negative (as assessed by conventional microscopy and sampling) to positive (as previously undetected micrometastases are revealed by way of these more sensitive detection methods). It is also well documented in the literature that the sensitivity of sentinel lymph node biopsy is improved by addition of immunohistochemical techniques to conventional microscopy.[3] Nevertheless, skip metastases may occur in the neck[4]; as a consequence, a detailed histologic, immunohistochemical, and molecular assessment of all the lymph nodes removed during a neck dissection is the most reliable currently available method of assessing the actual metastatic status of

TABLE 1. PATHOLOGICAL TECHNIQUES USED TO DETECT OCCULT LYMPH NODE METASTASES

- Traditional techniques
  - Routine histopathologic examination
  - Serial sections with hematoxylin and eosin
  - Histochemistry
- Sensitive techniques
  - Immunohistochemistry
    - On single section
    - On multiple sections
  - Molecular analysis

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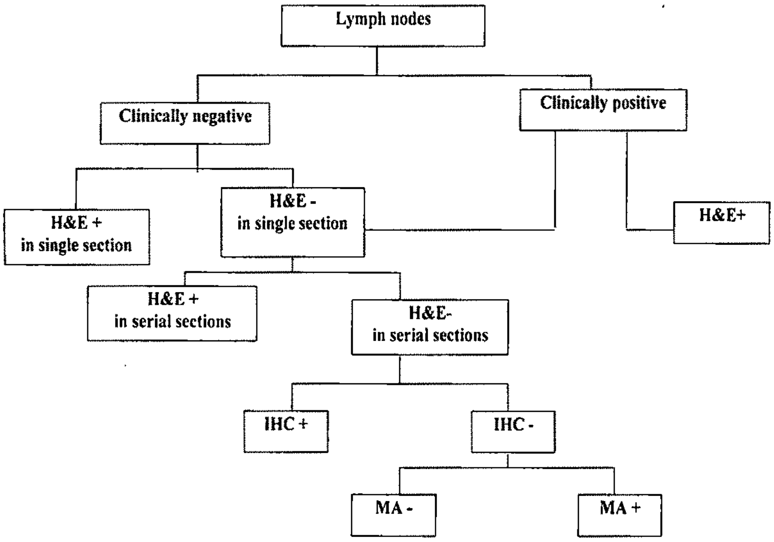

Algorithm of detection of micrometastases. H&E — hematoxylin and eosin, IHC — immunohistochemistry, MA — molecular analysis.

the lymph nodes.

In view of the fact that the presence or absence of lymph node metastases is the most important independent prognostic factor for predicting the outcome of patients with cancer of the head and neck, it is not surprising that there is a great deal of clinical interest in the detection of micrometastases. We must distinguish 1) tiny metastases (micrometastases) that are detected within regional nodes from cervical node dissection specimens that are already known from conventional microscopy to be clearly positive for metastatic disease from 2) those micrometastases that are uncovered within nodes from dissections that on conventional microscopy would have been designated as negative. While the former finding (micrometastases against a backdrop of grossly obvious metastases) is not likely to be significant, the latter case (micrometastases in the setting of otherwise negative node dissection specimens) may well have prognostic implications. As a general rule, micrometastases are defined as tumor deposits measuring 2 mm or less in greatest dimension.

EXPERIENCE WITH MICROMETASTASES IN OTHER TUMOR TYPES

Trojani et al,[5] using immunohistochemical techniques, discovered occult metastases in lymph nodes of patients affected by breast cancer. The presence of micrometastases was significantly correlated with a higher recurrence rate ($p = .0025$) and a lower overall survival ($p = .02$). The International (Ludwig) Breast Cancer Study Group[6] found an additional 9% of patients with micrometastases on examining 921 breast cancer patients "without" metastases in initial histologic evaluation of axillary lymph nodes. The patients with occult metastases had lower rates of disease-free survival (58% versus 74%; $p = .003$) and overall survival (79% versus 88%; $p = .002$) after 5 years' median follow-up, compared with patients whose lymph nodes remained negative on serial sectioning.

In 1997, Ishida et al[7] indicated that the presence of micrometastases in lymph nodes is an indispensable factor in determining the prognosis of gastric carcinoma patients. In 1997, O'Sullivan et al[8] found micrometastases in the bone marrow after "curative" surgery in about half of their patients with squamous esophageal cancer. Half of the patients with bone marrow micrometastases detected postoperatively had relapses within 18 months. In contrast, fewer than one fifth without bone marrow abnormalities developed recurrent disease.

Tumors classified as pN0 by routine methods could be reclassified more correctly as pN1 when the lymph nodes are examined immunohistochemically by means of a monoclonal antibody cocktail AE1/AE3 for cytokeratin and/or amplification of keratin 19 messenger RNA (K19) by reverse transcriptase–polymerase chain reaction.[1,3]

In 1998, Natsugoe et al[9] demonstrated that the presence of micrometastases with a stromal reaction in the regional lymph nodes from esophageal carcinomas is prognostically equivalent to the presence of metastases and that therefore the lymph nodes should be examined by sensitive immunohistochemical techniques to classify these patients correctly as pN1. The original pN1 patients, as expected, had a significantly shorter survival compared with pN0 patients, but most important, the new, "corrected" pN1 patients had the same shorter survival as the original pN1 cases ($p < .05$).

In 1999, Glickman et al[10] did not support that the presence of lymph node micrometastases in adeno-

TABLE 2. DETECTION OF LYMPH NODE MICROMETASTASES IN PATIENTS FOUND TO BE LYMPH NODE–NEGATIVE BY ROUTINE HISTOPATHOLOGIC EXAMINATION AND THEIR PROGNOSTIC SIGNIFICANCE

			Increased Detection			
Authors	*Year*	*Site*	*MSCHE*	*IHC*	*MA*	*Prognostic Significance*
Bussolati et al[21]	1986	Breast		24%		–
Trojani et al[5]	1987	Breast		14%		Yes
Friedman et al[14]	1988	Breast	9%			Yes
Sedmak et al[18]	1989	Breast		20%		Yes
International (Ludwig) Breast Cancer Study Group[6]	1990	Breast	9%			Yes
Schurman et al[22]	1990	Breast		37.5%		–
Chen et al[23]	1991	Breast		29%		Yes
de Mascarel et al[15]	1992	Breast		23%		Yes
Hainsworth et al[24]	1993	Breast		12%		Yes
Nasser et al[25]	1993	Breast	17%	14%		Yes, only for metastases >0.2 mm
Brennan et al[26]	1995	Head and neck			21%	–
Mori et al[27]	1995	Breast, gastrointestinal tract			54%	–
Noguchi et al[1]	1996	Breast			16%	–
Ishida et al[7]	1997	Stomach		18%		Yes
Izbicki et al[28]	1997	Esophagus		17%		Yes
Demeure et al[29]	1998	Pancreas			73%	Yes
Luketich et al[30]	1998	Esophagus			49%	Yes
Natsugoe et al[9]	1998	Esophagus		31.7%		Yes

MSCHE — multiple sectioning colored with hematoxylin and eosin, IHC — immunohistochemistry, MA — molecular analysis.

carcinoma and squamous cell carcinoma of the esophagus is an independent poor prognostic factor, but the follow-up of the patients was only approximately 2 years.

In 1999, Yokoyama et al[11] reported a study on the sensitivity and clinical implications of immunohistochemical detection of lymph node micrometastases in patients with gallbladder carcinoma. This study demonstrated a significant difference in postoperative survival between patients with lymph node micrometastases and those without ($p = .037$, log rank test). Among patients with no lymph node disease on routine histologic examination, there was a trend toward a worse survival rate in those with lymph node micrometastases ($p = .10$, log rank test).

Although contradictory results have been reported on the prognostic significance of micrometastases,[10,12-16] their presence is an independent predictor of decreased disease-free and/or overall survival, especially at 10 years.[6,11,17-20]

Table 2[1,5-7,9,14,15,18,21-30] summarizes the various techniques used by pathologists to detect micrometastases and their prognostic significance.

RELATION OF EXPERIENCE WITH MICROMETASTASES IN OTHER TUMORS TO HEAD AND NECK CANCER

While these studies in other tumor systems are certainly intriguing, suggesting (at least intuitively) that similar findings should be reproducible in the microcosm of head and neck cancers, the fact remains that hard empirical evidence of such a prognostic significance is yet to be accumulated. This, then, marks the territory of a fertile area for further clinical investigation. It seems likely that new molecular techniques and sensitive immunohistochemical investigations may allow us to identify micrometastases in clinically negative lymph nodes that may, in turn, have an impact on the staging and overall survival of head and neck cancer patients (particularly at the 10-year mark postdiagnosis). It just remains for patient data to confirm this.

CONCLUSIONS

There is, therefore, an abundance of evidence that the presence of micrometastases has clinical and prognostic implications. These findings argue in favor of selective neck dissections for head and neck cancers and indicates the importance of immunohistochemistry[31-33] and molecular biology[26,34] in correctly staging the neoplasms of these areas. Immunohistochemical and molecular staging are very attractive and interesting means to correctly classify these tumors. Accurate staging is essential to studies designed for the evaluation of postoperative adjuvant or therapeutic regimens. Many tumors are understaged even after surgical excision, and therefore, they should be restaged. The reliability of neck staging in clinically N0 cases is influenced by the pathological study of the specimen of the neck dissection, ie, by the number of sections investigated microscopically by the use of immunohistochemistry and molecular analysis. The dissection of 1 or 2 sentinel lymph nodes is not acceptable in head and neck cancers, given the frequency of "skip metastases" in the neck.[4]

REFERENCES

1. Noguchi S, Aihara T, Motomura K, Inaji H, Imaoka S, Koyama H. Histologic characteristics of breast cancers with occult lymph node metastases detected by keratin 19 mRNA reverse transcriptase–polymerase chain reaction. Cancer 1996; 78:1235-40.

2. Reintgen D, Shivers S. Sentinel lymph node micrometastasis from melanoma. Proven methodology and evolving significance [Editorial]. Cancer 1999;86:551-2.

3. Czerniecki BJ, Scheff AM, Callans LS, et al. Immunohistochemistry with pancytokeratins improves the sensitivity of sentinel lymph node biopsy in patients with breast carcinoma. Cancer 1999;85:1098-103.

4. Byers RM, Weber RS, Andrews T, McGill D, Kare R, Wolf P. Frequency and therapeutic implications of "skip metastases" in the neck from squamous carcinoma of the oral tongue. Head Neck 1997;19:14-9.

5. Trojani M, de Mascarel I, Bonichon F, Coindre JM, Delsol G. Micrometastases to axillary lymph nodes from carcinoma of breast: detection by immunohistochemistry and prognostic significance. Br J Cancer 1987;55:303-6.

6. International (Ludwig) Breast Cancer Study Group. Prognostic importance of occult axillary lymph node micrometastases from breast cancers. Lancet 1990;335:1565-8.

7. Ishida K, Katsuyama T, Sugiyama A, Kawasaki S. Immunohistochemical evaluation of lymph node micrometastases from gastric carcinomas. Cancer 1997;79:1069-76.

8. O'Sullivan GC, Collins JK, Kelly J, Morgan J, Madden M, Shanahan F. Micrometastases: marker of metastatic potential or evidence of residual disease? Gut 1997;40:512-5.

9. Natsugoe S, Mueller J, Stein HJ, Feith M, Höfler H, Siewert JR. Micrometastasis and tumor cell microinvolvement of lymph nodes from esophageal squamous cell carcinoma. Frequency, associated tumor characteristics, and impact on prognosis. Cancer 1998;83:858-66.

10. Glickman JN, Torres C, Wang HH, et al. The prognostic significance of lymph node micrometastasis in patients with esophageal carcinoma. Cancer 1999;85:769-78.

11. Yokoyama N, Shirai Y, Hatakeyama K. Immunohistochemical detection of lymph node micrometastases from gallbladder carcinoma using monoclonal anticytokeratin antibody. Cancer 1999;85:1465-9.

12. Huvos AG, Hutte RVP, Berg JW. Significance of axillary macrometastases and micrometastases in mammary cancer. Ann Surg 1971;173:44-6.

13. Fisher ER, Swamidoss S, Lee CH, Rockette H, Redmond C, Fisher B. Detection and significance of occult axillary node metastases in patients with invasive breast cancer. Cancer 1978; 42:2025-31.

14. Friedman S, Bertin F, Mouriesse H, et al. Importance of tumor cells in axillary node sinus margins ("clandestine" metastases) discovered by serial sectioning in operable breast carcinoma. Acta Oncol 1988;27:483-7.

15. de Mascarel I, Bonichon F, Coindre JM, Trojani M. Prognostic significance of breast cancer axillary lymph node micrometastases assessed by two special techniques: reevaluation with longer follow-up. Br J Cancer 1992;66:523-7.

16. Elson CE, Kufe D, Johnston WW. Immunohistochemical detection and significance of axillary lymph node micrometastases in breast carcinoma. A study of 97 cases. Anal Quant Cytol Histol 1993;15:171-8.

17. Rosen P, Saigo P, Braun D, Weathers E, Fracchia A, Kinne D. Axillary micro- and macrometastasis in breast cancer: prognostic significance of tumor size. Ann Surg 1981;194:585-91.

18. Sedmak D, Meineke T, Knetchgas D, Anderson J. Prognostic significance of cytokeratin positive breast cancer. Mod Pathol 1989;2:516-20.

19. McGuckin MA, Cummings MC, Walsh MD, Hohn BG, Bennett IC, Wright RG. Occult axillary node metastases in breast cancer: their detection and prognostic significance. Br J Cancer 1996;73:88-95.

20. Gerber B, Krause A, Reimer T. Immunohistochemically detected lymph-node micrometastasis in breast cancer and their correlation with prognostic factors. Breast J 1997;3:106-11.

21. Bussolati G, Gugliotta P, Morra I, Pierribiasi F, Berardengo E. Immunohistochemical detection of lymph node metastases from infiltrating lobular carcinoma of the breast. Br J Cancer 1986;54:631-6.

22. Schurman SH, Sharon N, Goldschmidt RA, Scanlon EF. Improved detection of metastases to lymph nodes and estrogen receptor determination. Arch Surg 1990;125:179-82.

23. Chen ZL, Wen DR, Coulson WF, Giuliano AE, Cochran AJ. Occult metastases in the axillary lymph nodes of patients with breast cancer node negative by clinical and histologic examination and conventional histology. Dis Markers 1991;9:239-48.

24. Hainsworth PJ, Tjandra JJ, Stillwell RG, et al. Detection and significance of occult metastases in node-negative breast cancer. Br J Surg 1993;80:459-63.

25. Nasser IA, Lee AKC, Bosari S, Saganich R, Heatley G, Silverman M. Occult axillary lymph node metastases in "node negative" breast carcinoma. Hum Pathol 1993;24:950-7.

26. Brennan JA, Mao L, Hruban RH, et al. Molecular assessment of histopathological staging in squamous-cell carcinoma of the head and neck. N Engl J Med 1995;332:429-35.

27. Mori M, Mimori K, Inoue H, et al. Detection of cancer micrometastases in lymph nodes by reverse transcriptase–polymerase chain reaction. Cancer Res 1995;55:3417-20.

28. Izbicki JR, Hosch SB, Pichlmeier U, et al. Prognostic value of immunohistochemically identifiable tumor cells in lymph nodes of patients with completely resected esophageal cancer. N Engl J Med 1997;337:1188-94.

29. Demeure MJ, Doffek KM, Komorowski RA, Wilson SD. Adenocarcinoma of the pancreas. Cancer 1998;83:1328-34.

30. Luketich JD, Kassis ES, Shriver SP, et al. Detection of micrometastases in histologically negative lymph nodes in esophageal cancer. Ann Thorac Surg 1998;66:1715-8.

31. van den Brekel MWM, Stel HV, van der Valk P, van der Waal I, Meyer CJLM, Snow GB. Micrometastases from squamous cell carcinoma in neck dissection specimens. Eur Arch Otorhinolaryngol 1992;249:349-53.

32. Ambrosch P, Brinck U. Detection of nodal micrometastases in head and neck cancer by serial sectioning and immunostaining. Oncology (Huntingt) 1996;10:1221-9.

33. Enepekides DJ, Sultanem K, Nguyen C, Shenouda G, Black MJ, Rochon L. Occult cervical metastases: immunoperoxidase analysis of the pathologically negative neck. Otolaryngol Head Neck Surg 1999;120:713-7.

34. Brennan JA, Sidransky D. Molecular staging of head and neck squamous carcinoma. Cancer Metastasis Rev 1996;15:3-10.

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FIFTH INTERNATIONAL CONFERENCE ON HEAD AND NECK CANCER

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BOOK REVIEWS

Allergy in ENT Practice, A Basic Guide

Houston C. King, Richard L. Mabry, and Cynthia S. Mabry. Soft cover, illustrated, indexed, 452 pages, 1998. Thieme, New York, NY, $59.

Purpose: To provide an introduction for physicians and nurses in any field or specialty of medicine to the importance of recognizing allergic disease in their patients, as well as to provide a basic guide for setting up a testing laboratory in the office. The book addresses how to recognize the allergic patient and discusses the underlying physiological principles of allergic disease and treatment modalities, including environmental control, pharmacotherapy, and allergenic extract injection immunotherapy. For those so inclined, the book is an excellent source of aid in setting up an allergy department.

Contents: The text is extremely well organized and very easy to follow, from consideration of setting up an allergy practice through trouble-shooting common problems that anyone treating the allergic patient would come up against.

Attributes: The book is exceptionally well written and easy to follow and filled with a variety of illustrations to enhance specific points. The "Nurse's Notes" that are found throughout the textbook make it an excellent reference and "must" reading for any nurse or technician engaged in the management of the allergic patient. The 5 appendices provide the novice as well as the experienced practitioner with valuable information to aid in the management of patients, including what primary and secondary allergens one should suspect in specific geographic areas of the country. This appendix is also a great reference for the practitioner to use in selecting what allergens to test in his or her geographic region of the country.

Limitations: The only limitation in this text is an editing error noted on page 125, involving preparation of the histamine control. Until approximately 1994, the standard histamine control that was used was a 2.75 mg/5 mL solution that was serially fivefold diluted to a #3 dilution. The manufacturer of that particular product discontinued it, and following that, a variety of different concentrations of histamine base became available. Due to an error that got past the proofreading, the incorrect dilution level is listed for histamine, but the principle of the calculation for the histamine control is correct. This should not make any difference, since histamine is now available in the 0.275-mg/mL strength, as opposed to 0.0275 mg/mL.

Applicability: This text is invaluable for any physician of any specialty and for nurses and technicians involved in the management of allergic patients. It is a basic reference guide to safely identifying, testing, and treating allergic patients and is exceptionally well written and extremely easy to follow in its layout. The various graphics and Nurse's Notes throughout the text are extremely useful in augmenting specific points.

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Managed Care, Outcomes, and Quality. A Practical Guide

Steven F. Isenberg, editor. Soft cover, illustrated, indexed, 343 pages, 1998. Thieme, New York, NY, $59.

Purpose: Offers an up-to-date guide to the features and intricacies of managed care.

Contents: Section I includes a rundown of capitation, reimbursement in a managed care environment, CPT issues, and the idiosyncrasies of a managed care environment. Section II describes outcomes research and useful information to implement such in office practices. In Section III, one learns of the improvements and downsizing adopted as a response to the need for cost-cutting efficiency. Section IV describes the physician's struggle to respond to managed care, eg, networks, mergers, and acquisitions.

Attributes: The text is a complete reference book that provides the head and neck physician with guidelines, though not clear solutions, to the problems of managed care. Insights are given into managed care plans that are playing an increasingly important role in physicians' practices, in terms of data they possess, analyses they do, and so-called incentives they provide. Above all, the book tackles the economics of managed care: capitation, legal issues, physician organizations, contract profitability, termination, etc.

Limitations: Certain parts of the book (Preface, Foreword, and Chapter 23) provide compelling arguments that answers to this great dilemma in medicine reside in managed care. Not every reader will relish the description of managed care and health maintenance organizations as "free market" alternatives.

Applicability: The book gives a comprehensive and thorough overview of the possibilities and limitations of managed care. It is recommended to all otolaryngologists.

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Endoscopy in Neuro-otology

Jacques Magnan and Mario Sanna. Hard cover, illustrated, indexed, 101 pages, 1999. Thieme, New York, NY, $198.

Purpose: Application of endoscopy in the field of neuro-otology. Otologic endoscopy, such as in the involvement of the middle ear, is not included in this text.

Contents: There are 3 parts to the book: 1) endoscope technology, 2) endoscopic anatomy of the cerebellopontine angle and adjacent structures, and 3) microscopic and endoscopic neuro-otological surgery. The second chapter shows details of neural and vascular anatomy that may not be available in microscopic surgery. The third chapter deals with the advantage of endoscopy as the main technique or as an adjunct to microscopic surgery in difficult surgical encounters.

Attributes: In this atlas we learn the workings of endoscopy as a major investigative and therapeutic technology in the disciplines of neuro-otology and skull base surgery. It succeeds in showing the fundamental importance of the endoscopic procedure, ie, it allows a more comprehensive map of the neurovascular components to be obtained without the need for cerebellum retraction, not to mention the important contribution to a minimally invasive surgery approach that offers patients the potential for minimal morbidity. Most impressive are the excellent illustrations throughout the text.

Limitations: One wonders if the difficulties and obstacles mentioned by the authors for endoscopic vestibular neurectomy do not apply throughout all the procedures. These are 1) insufficient spontaneous retraction of brain structures, 2) presence of thick arachnoid wrappings requiring dissection, 3) presence of vascular loops hindering direct access to structures, and 4) difficulty in determining with certainty, through the scope, whether surgery is complete.

Applicability: The text is a valuable reference for the neuro-otologist.

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